Biosafety Guidelines for Research Laboratories

Ministry of Public Health

Department of Research

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Contents

Preface: 14

Purpose....14

Policy Aim and Objectives 14

General principles 15

Introduction 15

Table 1. Classification of infective microorganisms by risk group 15

Table 2. Relation of risk groups to biosafety levels, practices and equipment 16

Table 3. Summary of facility biosafety level requirements 18

PART I 19

Biosafety guidelines 19

1. Microbiological risk assessment 20

Table 4. Pathogen Risk Assessment....23

WHO Biorisk management - Laboratory biosecurity guidance - September 2006....24

Specimens for which there is limited information 24

Immunization of staff 25

Risk assessment of genetically modified microorganisms 25

2. Containment Laboratory – Biosafety Level 2 26

Code of practice 26

Access....27

Figure 1. Biohazard warning sign for laboratory doors 27

Personal Protective Equipment 27
Standard Microbiological Practices....28
Laboratory work areas....29
Biosafety management....29
Laboratory design and facilities 30
Design features 30

Figure 2. Typical BSL-1 laboratory....30

Figure 3. Typical BSL-2 laboratory....30

Laboratory equipment....32
Essential biosafety equipment 33
Health and medical surveillance 33
Guidelines for surveillance of laboratory workers handling microorganisms at Biosafety Level 1....34
Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 2 34
Training 34
Waste handling....35
Decontamination 35
Handling and disposal procedures for contaminated materials and wastes 36
Sharps....36
Contaminated (potentially infectious) materials for autoclaving and reuse 36
Contaminated (potentially infectious) materials for disposal....37
Chemical, fire, electrical, radiation and equipment safety 37

3. High Containment Laboratory – Biosafety Level 3....38

Code of practice 38

Laboratory design and facilities 39

Figure 4. An example of laboratory design for Biosafety Level 3 40

Laboratory equipment....41
Health and medical surveillance 41

Figure 5. Suggested format for medical contact card 42

4. Maximum Containment Laboratory – Biosafety Level 4....43

Code of practice 43

Laboratory design and facilities 43

1. Primary containment. 44

2. Controlled access 44
3. Controlled air system 44
4. Decontamination of effluents....45
5. Sterilization of waste and materials....45
6. Airlock entry ports 45
7. Emergency power....45
8. Containment drain(s) 46
5. Laboratory animal facilities....47
Table 5 Animal facility containment levels: summary of practices and safety Equipment 47
Animal facility – Animal Biosafety Level 1....48
Animal facility – Animal Biosafety Level 2....48
Animal facility – Animal Biosafety Level 3....49
Animal facility – Animal Biosafety Level 4....50
Invertebrates 51
6. Guidelines for laboratory/facility commissioning 53
7. Guidelines for laboratory/facility certification....56
PART II....58
Laboratory biosecurity 58
8. Laboratory biosecurity concepts....59
PART III....61
Laboratory equipment 61
9. Biosafety cabinets....62

Table 6. Selection of biosafety cabinet (BSC) by type of protection need.....63

Table 7. Differences between Class I, II and III biosafety cabinets (BSCs) 64

Selection of a biosafety cabinet.....64

1. What needs to be protected?.....64
2. What are all of the different types of work to be done in the cabinet? 65
3. What types and quantities of chemical vapors will be generated in the BSC?.....65
4. Is there an appropriate location for the cabinet ductwork? 66
5. If the volume of air being removed by the BSC's exhaust system is reduced or eliminated, due to malfunction, what is its effect on BSC performance and what is preferred by the user? 67

Class I biosafety cabinet.....68

Figure 6. Schematic diagram of a Class I biosafety cabinet. 68

Class II biosafety cabinet.....68

Class II Type A1 biosafety cabinets:.....69
Class II Type A2 biosafety cabinets:.....69

Figure 7. Schematic diagram of a Class II, Type A1 and A2 biosafety cabinets 70

Figure 8. Schematic diagram of a Class II, Type A1 and A2 biosafety cabinet canopy exhaust.....71

Class II Type B1 biosafety cabinets:.....72

Figure 9. Schematic diagram of a Class II, Type B1 biosafety cabinet 73

Class II Type B2 biosafety cabinets:.....74

Figure 10. Schematic diagram of a Class II, Type B2 biosafety cabinet 75

Class III biosafety cabinets:.....76

Figure 11. Schematic representation of a Class III biosafety cabinet 77

Using biosafety cabinets in the laboratory 77

Laboratory location of biosafety cabinets.....77

Figure 12. Suggested Laboratory Locations for Class II Biosafety Cabinets 78

Operations....79
Material placement....79
Type A and Type B BSC shutdown....79
BSC start up procedure....80
Ultraviolet lights....81
Open flames....81
Spills....82
Certification....82
Surface cleaning and disinfection....82

Suggested surface disinfectants....83

BSC space decontamination....84
Personal protective equipment....85
Alarms....85

10. Safety equipment....86

Table 8. Biosafety equipment....86

Negative-pressure flexible-film isolators....87

Pipetting aids....88

Homogenizers, shakers, blenders and sonicators....89

Disposable transfer loops....89

Micro incinerators....89

Personal protective equipment and clothing....89

Table 9. Personal protective equipment....90

Laboratory coats, gowns, coveralls, aprons....90
Goggles, safety glasses, face shields, face masks....91
Respirators....92
Gloves....92

....94

PART IV....94

Standard microbiological practices....94

11. Laboratory techniques....95

Safe handling of specimens in the laboratory 95

Specimen containers 95
Transport of specimens within the facility 95
Receipt of specimens 95
Opening packages 95

Use of pipettes and pipetting aids....96

Avoiding the dispersal of infectious materials 96

Use of biosafety cabinets (BSCs) 97

Avoiding ingestion of infectious materials and contact with skin and eyes 98

Avoiding injection of infectious materials 98

Separation of serum 98

Use of centrifuges 99

Use of homogenizers, shakers, blenders and sonicators....100

Use of tissue grinders....100

Care and use of refrigerators and freezers 100

Opening ampoules containing lyophilized infectious materials....101

Storage of ampoules containing infectious materials 101

Standard precautions with blood and other body fluids, tissues and excreta 101

Collection, labeling and transport of specimens....102
Opening specimen tubes and sampling contents....102
Glass and “sharps” 102
Films and smears for microscopy 102
Automated equipment (sonicators, vortex mixers) 103
Tissue sectioning....103
Decontamination 103
Precautions with materials that may contain prions 103

12. Contingency plans and emergency procedures....106

Contingency plan....106

Emergency procedures for microbiological laboratories 107

Puncture wounds, cuts and abrasions 107
Ingestion of potentially infectious material 107
Potentially infectious aerosol release (outside a biosafety cabinet) 107
Broken containers and spilled infectious substances 108
Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets 108
Breakage of tubes inside sealable buckets (safety cups) 109
Fire and natural disasters 109
Emergency services: whom to contact 109
Emergency equipment 110

13. Disinfection and sterilization 111

Definitions 111

Antimicrobial 111
Antiseptic 111
Biocide 111
Chemical germicide 111
Decontamination 111
Disinfectant 111
Disinfection 112
Microbicide 112
Sporicide 112
Sterilization 112

Cleaning laboratory materials 112

Chemical germicides 112

Table 10. Recommended dilutions of chlorine-releasing compounds 113

Chlorine (sodium hypochlorite) 113
Sodium dichloroisocyanurate 114
Chloramines 114
Chlorine dioxide 115
Formaldehyde 115
Glutaraldehyde 116
Phenolic compounds 116
Quaternary ammonium compounds 117
Alcohols 117
Iodophors and Iodine 118
Hydrogen peroxide and peracids 118

Local environmental decontamination 119

Decontamination of biosafety cabinets 119

Hand-washing/hand decontamination 120

Heat disinfection and sterilization 121

Autoclaving 121

Figure 13. Gravity displacement autoclave 122

Incineration 124
Superheated water grinding 124
Alkaline hydrolysis 124
Disposal 125

14. Introduction to the transport of infectious substances 126

International transport regulations 126

Classes of Dangerous Goods 127

Class 6, Division 6.1 Toxic Substances 127

Class 6, Division 6.2 Infectious Substances 127

The basic triple packaging system 128

Figure 14. Packing and labeling of Category A Infectious Substances 129

Figure 15. Packing and labeling of Category B Biological Substances 129

Spill clean-up procedure 130

..... 131

PART V 131

Introduction to biotechnology 131

15. Biosafety and recombinant or synthetic nucleic acid technology 132

Biosafety considerations for biological expression systems 132

Biosafety considerations for expression vectors 133

Viral vectors for gene transfer 133

Transgenic and “knock-out” animals.....133

Transgenic plants.....134

Risk assessments for genetically modified organisms 134

Hazards arising directly from the inserted gene (donor organism) 134
Hazards associated with the recipient/host.....135
Hazards arising from the alteration of existing pathogenic traits 135

PART VI.....136

Chemical, fire and electrical safety.....136

16. Hazardous chemicals.....137

Routes of exposure 137

Storage of chemicals.....137

General rules regarding chemical incompatibilities.....137

Toxic effects of chemicals 137

Table 11. General rules for chemical incompatibilities.....138

Explosive chemicals.....138

Chemical spills 139

Compressed and liquefied gases 140

Table 12. Storage of compressed and liquefied gases.....140

17. Additional laboratory hazards.....141

Fire hazards.....141

Table 13. Types and uses of fire extinguishers 142

Electrical hazards.....142

Noise 142

Ionizing radiation 143

Principles of ionizing radiation protection 143
Safe practices for work with radionuclides 144

Figure 16. International radiation hazard symbol 145

PART VII 147

Safety organization and training 147

18. The biosafety officer and biosafety committee 148

Biosafety officer 148

Biosafety committee 149

19. Safety for support staff 151

Engineering and building maintenance services 151

Cleaning (domestic) services 151

20. Training programs 152

PART VIII 153

Safety checklist 153

21. Safety checklist 154

Laboratory premises 154

Storage facilities 154

Sanitation and staff facilities 155

Heating and ventilation 155

Lighting 155

Services 155

Laboratory biosecurity 156
Fire prevention and fire protection 156
Flammable liquid storage 157
Compressed and liquefied gases 157
Electrical hazards 158
Personal protection 158
Health and safety of staff 159
Laboratory equipment 159
Infectious materials 160
Chemicals and radioactive substances 160
MOPH Laboratory Inspection Checklist Date: 161
PART IX 171
References and Annexs 171
References 172
ANNEX 1 - First aid 176
The first aid box - hospital 176
The first aid box - workplace 177
ANNEX 2 - Immunization of staff 178
ANNEX 3 - Equipment safety 179
Equipment that may create a hazard 179
Table A3-1. Equipment and operations that may create hazards 179
Table A3-2. Common causes of equipment-related accidents 182

ANNEX 4 - Human and Animal etiological agents 183

Table A4-1. Basis for the Classification of Biohazardous Agents by Risk Group (RG) 183

United States.....183

Select agents.....184

Table A4-2. Select Agents 184

Canada 186

ANNEX 5 - Chemicals: hazards and precautions.....188

Table A5-1. Chemicals: hazards and precautions 189

ANNEX 6 – Biomedical Research Acronyms 214

Preface:

Purpose

This biosafety manual provides guidelines to be followed by all research laboratories in the State of Qatarto maintain the high research standards of the Ministry of Public Health (MOPH).

Policy Aim and Objectives

Thisbiosafety manual isintended to ensure that biomedical research personnel and laboratories in Qatar have state-of-the-art facilities and training that conform to international standards of research practices.

Adverse events and unanticipated problems must be reported in a timely, meaningful way. Review of adverse events by the biosafety committee may result in changes to practices or facility design to reduce future hazards, loss of research time and financial burden to the facility.

In order to achieve these goals, the manual will address:

1. Biosafety guidelines.
2. Laboratory biosecurity.
3. Laboratory equipment.
4. Standard microbiological practices.
5. Introduction to biotechnology.
6. Chemical, fire and electrical safety.
7. Safety organization and training.
8. Safety checklists.
9. Reporting of unanticipated problems involving risks to personnel or community.
10. References and annexes.

During development of these guidelines, the Ministry of Public Health consulted with guidelines developed by international organizations. A list of references is provided at the end of this document. The MOPH biosafety manual incorporates information from the World Health Organization (WHO)Laboratory Biosafety Manual, Third Edition (1); an accepted international model that could be tailored to Qatar's research standards. Additional information was derived from the 1997 WHO publication Safety in Health-Care Laboratories (3), NIH/CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition (8),NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) (2) and NSF/ANSI 49 - Biosafety Cabinetry: Design,Construction, Performance, andField Certification. (9)

General principles

Introduction

Throughout this manual, references are made to the relative hazards of infective microorganisms by risk group (WHO Risk Groups 1, 2, 3 and 4). This risk group classification is to be used for laboratory work only. Table 1 describes the risk groups.

Table 1. Classification of infective microorganisms by risk group

Risk Group 1 (RG1)	Agents not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Research laboratory facilities are designated as:

1. Biosafety Level 1 (basic laboratory).
2. Biosafety Level 2 (containment laboratory).
3. Biosafety Level 3 (high containment laboratory).

4. Biosafety Level 4 (maximum containment laboratory).

Biosafety level designations are based on a composite of laboratory design features, primary containment equipment, laboratory practices and operational procedures required for work with biological materials from the various risk groups (see Annex 4). Table 1 relates but does not "equate" risk groups to laboratory biosafety levels. Specific operational procedures with a particular risk group biological material may require a higher level of laboratory containment than shown in Table2.

Table 2. Relation of risk groups to biosafety levels, practices and equipment

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1.	Basic Biosafety Level 1	Basic teaching, research	Standard microbiological practices (SMP)	None; open bench work
2.	Containment BiosafetyLevel 2	Primary health services; diagnostic services, research	SMP plus protective clothing and gloves, biohazard sign, annual pathogen training, biosafety manual, spills and exposures reported	BSC for most activities, self-closing door with lock, handwashing sink near exit, vacuum line HEPA protection, directional inward airflow and no air recirculation
3.	High Containment Biosafety Level 3	Special diagnostic, services, research	As Level 2 plus controlled anteroom access, special clothing, annual BSL-3 pathogen training, treat contaminated material in facility	BSC and/or other primary containment devices for all activities, dedicated lab air exhaust system with HEPA filtration, autoclave available, laboratory can be sealed for space decontamination

4.	Maximum ContainmentBiosafety Level 4	Dangerous pathogen	As Level 3 plus special clothing, clothing change before entering, shower before exit, all material decontaminated before removal from facility, annual BSL-4 training	Class III BSCs or positive pressure suits with Class II BSCs, separate building or isolated zone, airlock entry, airlock shower exit, dedicated HEPA filtered supply air, special waste disposal, double-ended autoclave and dip tanks through the wall

BSC, biosafety cabinet; SMP, standard microbiological practices (see Part IV of this manual)

The assignment of biological material to a biosafety level for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate biosafety level. For example, a microorganism that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices and procedures for safe conduct of work. However, if particular experiments require the generation of high-concentration aerosols, then Biosafety Level 3 may be more appropriate to provide the necessary degree of safety, since it ensures superior containment of aerosols in the laboratory workplace. The biosafety level assigned for the specific work to be done is therefore driven by professional judgement based on a risk assessment, rather than by automatic assignment of a laboratory biosafety level according to the particular risk group designation of the biological material to be used.Table 3 summarizes the facility requirements at the four biosafety levels.

Table 3. Summary of facility biosafety level requirements

	BIOSAFETY LEVEL			
	1	**2**	**3**	**4**
Isolation of laboratory (a)	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
Inward airflow	No	Yes	Yes	Yes
Controlled ventilating system	No	Yes	Yes	Yes
HEPA-filtered air exhaust	No	No	Yes	Yes
Double-door entry	No	No	Yes	Yes
Airlock with showers	No	No	No	Yes
Anteroom	No	No	Yes	---
Anteroom with shower	No	No	Yes/No (b)	No
Effluent treatment	No	No	Yes/No (b)	Yes
Autoclave:				
On site	No	Desirable	Yes	Yes
In laboratory room	No	No	Desirable	Yes
Double-ended	No	No	Desirable	Yes
Biosafety cabinets	No	Yes	Yes	Yes
Personnel safety monitoring capability (c)	No	No	Desirable	Yes

a) Environmental and functional isolation from general traffic.

b) Dependent on agent(s) used in the laboratory.

c) For example, window, closed-circuit television, two-way communication.

Thus, the assignment of a biosafety level takes into consideration the biological material (pathogenic microorganism, biological toxin), worker training, type of research (basic, clinical, large scale, production), use of research animals, primary containment equipment, and research procedures.

PART I

Biosafety guidelines

1. Microbiologicalrisk assessment

The backbone of the practice of biosafety is risk assessment. While there are manytools available to assist in the assessment of risk for a given procedure or experiment, the most important component is professional judgement and experience. Risk assessments shouldbe performed by the individuals most familiar with the specific characteristics of theorganisms being considered for use, the equipment and procedures to be employed,animal models that may be used and the containment equipment and facilitiesavailable. The laboratory director or principal investigator is responsible for ensuringthat adequate and timely risk assessments are performed and for working closely withthe institution's safety committee and biosafety personnel to ensure that appropriateequipment and facilities are available to support the work being considered.

Onceperformed, risk assessments should be reviewed routinely and revised when necessary,taking into consideration the acquisition of new data having a bearing on the degreeof risk and other relevant new information from the scientific literature.

One tool for performing a microbiological risk assessmentis the listing of risk groups for microbiological agents. However,simple reference to the risk grouping for a particular microorganism is insufficient in the conductof a risk assessment. Other factors that should be considered, as appropriate, include:

1. Pathogenicity of the microorganism and infectious dose.
2. Potential outcome of exposure.
3. Natural route of infection.
4. Other routes of infection resulting from laboratory manipulations (parenteral,airborne, ingestion).
5. Stability of the microorganism in the environment.
6. Procedures for concentrating the microorganism and volume of concentrated material to be manipulated.
7. Presence of a suitable host (human or animal).
8. Information available from animal studies and reports of laboratory-acquiredinfections or clinical reports.
9. Planned laboratory procedures such as sonication, filtration, centrifugation, etc.
10. Any genetic manipulation of an organism that may extend the host range of themicroorganism or alter the microorganism's sensitivity to known, effective treatment regimens.
11. Local availability of effective prophylaxis or therapeutic interventions.

Risk assessments encompass five main elements:

1. Hazard identification.
2. Exposure assessment.
3. Dose-response assessment.
4. Risk characterization.
5. Risk management (job analysis).

Risk assessment team members may include:

1. Investigator/scientist.
2. Laboratory staff.
3. Animal care staff, when appropriate.
4. Animal veterinarian, when appropriate.
5. Plant pathogen or plant pest containment expert, when appropriate.
6. Occupational health and biosafety professionals.

Risk assessment hazards considered are:

1. Animal hazards.
2. Microorganism/pathogen/recombinant hazards.
3. Chemical hazards.
4. Radiological hazards.
5. Physical hazards.

Microorganism/pathogen/recombinant's factors associated with risk of disease or injury are:

1. Virulence.
2. Infectious dose.
3. Route of infection (portal of entry).
4. Toxigenicity.
5. Microorganism's host range.
6. Whetherthe microorganism is endemic or exotic to the local environment.
7. Availability of effective preventive measures.
8. Availability of effective treatment.

Factors associated with a worker's risk of exposure are:

1. Worker's work activity; diagnostic, research or production scale.
2. Worker's proficiency, attitude and safety awareness.
3. Worker's age, sex, pregnancy, race, immune status and medications.

Risk management plan should include:

1. Biosafety containment level assignment to the facility.
2. Microbiological practices.
3. Safety equipment.
4. Engineering controls.
5. Personal protective equipment.
6. Work practices.
7. Standard Operating Procedures (SOPs).
8. Emergency procedures.
9. Work schedule.
10. Calendar of work days.
11. Investigation protocols that include all risk management plans.

Investigation protocol review includes:

1. Committee (biosafety, human subjects and animal subjects review, as appropriate.
2. Meetings with workers to discuss approved protocols.
3. Worker training.
4. Dry runs without microorganism/pathogen/recombinant.
5. Regular audits.

Table 4presents a table that can be used to assess pathogen risks.

Table4.Pathogen Risk Assessment

Risk Factors	Risk Assessment Level	
	<Decrease	>Increase
Pathogen Disease Potential		
Known, classified		
Suspected, classified		
Known, unclassified		>>>
Unknown		>>>>
Pathogen Aerosol Potential		
Tissue procedure	<<<	
Culture procedure		>>>
Concentration procedure		>>>>>>
Animal/non-shedder	<<<	
Animal/shedder		>>>>>>
Pathogen Infectious route		
Respiratory		>>>>>>
Mucous membrane		>>>
Parenteral	<<<	
Other	<<<	
Disease Severity		
Moderate		>>
Severe		>>>
Life threatening/lethal		>>>>>>>>
Disease Prophylaxis		
None		>>>>>>>>
Vaccine	<<	
Immune globulin	<<<	
Antibiotics	<<<	
Antivirals	<<<	
Other Factors		
Livestock pathogen		>>>
Poultry pathogen		>>>

(NSF/ANSI 49-2014 table kindly provided by NSF International, Ann Arbor, MI, USA)

On the basis of the information ascertained during the risk assessment, a biosafety level can be assigned to the planned work, appropriate personal protective equipment selected

and standard operating procedures (SOPs) incorporating other safety interventions to ensure the safest possible conduct of the work implemented.

WHO Biorisk management - Laboratory biosecurity guidance - September 2006

This document introduces the overarching "biorisk management" approach that hasresulted from careful thinking, comprehensive study of prevailing practices andrecommendations, review of international norms and standards and relevant ethicalconsiderations. Shortcomings currently observed in a number of settings are discussedand practical solutions are proposed.
http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf

Specimens for which there is limited information

The risk assessment procedure described above works well when there is adequate information available. However, there are situations when the information is insufficient to perform an appropriate risk assessment, such as clinical specimens or epidemiological samples collected in the field. In these cases, it is prudent to take a cautious approach to specimen manipulation.

1. Standard precautions (21) should always be followed and barrier protections applied (gloves, gowns, eye protection) whenever samples are obtained from patients.
2. Biosafety Level 2 practices and procedures should be the minimum requirement for handling specimens.
3. Transport of specimens should comply with national and/or international rules and regulations.

Some information may be available to assist in determining the risk of handling these specimens:

1. Medical data about the patient.
2. Epidemiological data (morbidity and mortality data, suspected route of transmission and other outbreak investigation data).
3. Information on the geographical origin of the specimen.

During outbreaks of disease of unknown etiology, appropriate ad hoc guidelines may be generated and posted by national competent authorities and/or WHO on the internet (as was the case during the 2003 emergence of the severe acute respiratory

syndrome (SARS) outbreak) to indicate how specimens should be consigned for shipment and the biosafety level at which they should be analyzed.

Immunization of staff

The risks of working with particular biological materials should be fully discussed with individual workers. The local availability, licensing state and utility of possible vaccines and/ or therapeutic drugs (e.g. antibiotic treatments) in case of exposure should be evaluated before work with such materials is started. Some workers may have acquired immunity from prior vaccination or infection.

If a particular vaccine or toxoid is locally licensed and available, it should be offered after a risk assessment of possible exposure and a clinical health assessment of the individual has been performed.

Laboratory staff shall be informed about facilities for specific clinical case management following accidental exposures.

Risk assessment of genetically modified microorganisms

A detailed risk assessment discussion of genetically modified organisms (GMOs) is provided in Chapter 15.

2. Containment Laboratory – Biosafety Level 2

For the purposes of this manual, the guidance and recommendations given as minimum requirements pertaining to laboratories of all biosafety levels are directed at microorganisms in Risk Groups 1–4. Although some of the precautions may appear to be unnecessary for some organisms in Risk Group 1, they are desirable for training purposes to promote standard (i.e. safe) microbiological practices.

Research laboratories must all be designed for Biosafety Level 2 or above. As no laboratory has complete control over the specimens it receives, laboratory workers may be exposed to organisms in higher risk groups than anticipated. This possibility must be recognized in the development of safety plans and policies. Accreditation of clinical laboratories is required.

Code of practice

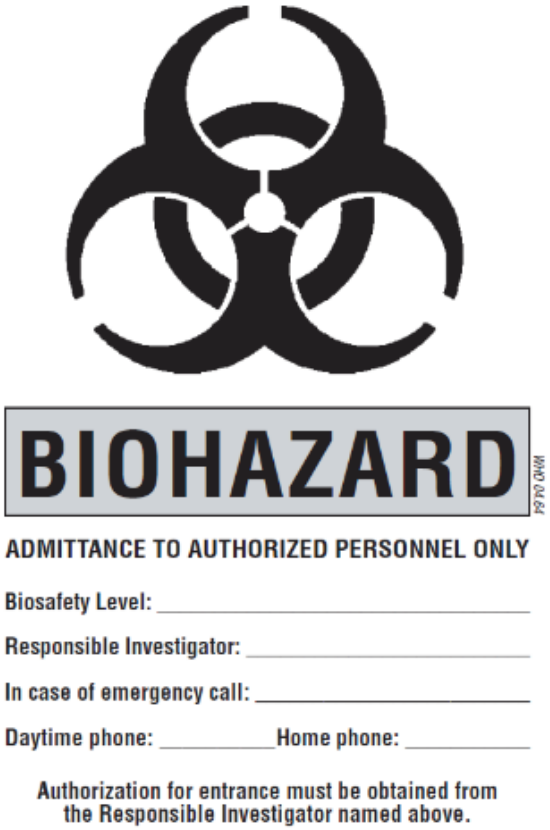
This code is a listing of the most essential laboratory practices and procedures that are basic to standard microbiological practices. In many laboratories and national laboratory programs, this code may be used to develop written practices and procedures for safe laboratory operations.

Each laboratory should adopt a safety or operations manual that identifies known and potential hazards and specifies practices and procedures to eliminate or minimize such hazards. Standard microbiological practices are fundamental to laboratory safety. Specialized laboratory equipment and engineering controls are a supplement to, but can never replace, appropriate procedures. The most important concepts are listed below.

Access

1. The international biohazard warning symbol and sign (Figure 1) must be displayed on the doors of the rooms where microorganisms of Risk Group 2 or higher risk groups are handled.

Figure 1. Biohazard warning sign for laboratory doors



2. Only authorized persons shall be permitted to enter the laboratory working areas.
3. Laboratory doors should be kept closed.
4. Children shall not be authorized or allowed to enter laboratory working areas.
5. Access to animal rooms shall be specially authorized.
6. No animals shall be allowed to enter the laboratory except those involved in the work of the laboratory.

Personal Protective Equipment

1. Laboratory coveralls, gowns or uniforms must be worn at all times when working in the laboratory.

2. Appropriate gloves (long sleeve nitrile gloves are recommended) must be worn for all procedures that may involve direct or accidental contact with blood, body fluids, other potentially infectious materials or infected animals. After use, gloves shall be removed aseptically and hands must then be washedwith mild, non-antiseptic soap in warm running water for 20-30 seconds.
3. Personnel must wash their hands after handling infectious materials, animals and before leaving laboratory work areas.
4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear personal protective equipment andlaboratory clothing outside the laboratory, e.g. in canteens, coffee rooms, offices, libraries, staff rooms and toilets.
6. Open-toe footwear must not be worn in laboratories.
7. Eating, drinking, chewing, smoking, applying cosmetics and handling contact lenses is prohibited in laboratory work areas.
8. Storing human food and drinks anywhere in laboratory work areas is prohibited.
9. Protective laboratory clothing that has been worn in the laboratory must not be stored in the same lockers or cupboards as street clothing.
10. Protective laboratory clothing that has not been contaminated may be stored near the laboratory exit.
11. Laboratory gowns or coats that may have become contaminated must be placed in a soiled laundry container and be laundered by the institution.

Standard Microbiological Practices

1. Tie back long hair.
2. Do not wear dangling jewelry.
3. Do not bring food, gum, drinks (including water), or water bottles into the laboratory.
4. Do not touch the face, apply cosmetics, adjust contact lenses, or bite nails.
5. Do not handle personal items (cosmetics, cell phones, calculators, pens, pencils, etc.) while in the laboratory.
6. Pipetting by mouth is strictly forbidden.
7. Materials in the laboratory must not be placed in the mouth. Labels must not be licked.
8. All technical procedures shall be performed in a way that minimizes formation of aerosols or droplets.
9. Use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals or septum bottles or tubes.

10. All spills, accidents and overt or potential exposures to infectious materials must be reported immediately to the laboratory supervisor. A written record of such accidents and incidents shall be maintained.
11. A written procedure for the spill clean-up must be developed and followed.
12. Potentially infectious materials or culture liquids must be decontaminated (chemically or physically) before discharge into the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the material(s) being handled.
13. Written documents and notes that will be removed from the laboratory must be protected from contamination while they are in the laboratory.

Laboratory work areas

1. The laboratory shall be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially infectious material, after each task and at the end of the workday.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation of potentially infectious materials must follow applicable national and/or international regulations.
5. When windows must be opened, they should be fitted with arthropod-proof screens.

Biosafety management

1. The laboratory director (the person who is in charge of the laboratory) is responsible for ensuring the development and adoption of a biosafety management plan and a safety or operations manual.
2. The laboratory supervisor (reporting to the laboratory director) shall ensure that regular laboratory safety training is provided to the laboratory personnel.
3. Laboratory personnel shall be advised of special hazards and required to document that they have read the safety or operations manual that shall be available in the laboratory. The laboratory supervisor shallensure that all personnel understand the laboratory policies and procedures.
4. There shall be a laboratory arthropod and rodent control program.
5. Appropriate medical evaluation, surveillance and treatment shall be provided to all laboratory personnel.Personnel medical records shall be maintained by the occupational health department.

Laboratory design and facilities

Conditions that increase hazards to research personnel must be considered when designing a research facility. These include:

1. Procedures that generate aerosols.
2. Work with large volumes and/or high concentrations of microorganisms.
3. Overcrowding of work spaces with materials and equipment.
4. Infestation with rodents and arthropods.
5. Unauthorized entrance of personnel.
6. Work with radionuclides, chemicals, toxins and potentially infectious materials.

Design features

Examples of laboratory designs for Biosafety Levels 1 and 2 are shown in Figures 2 and 3, respectively.

Figure 2. Typical BSL-1 laboratory

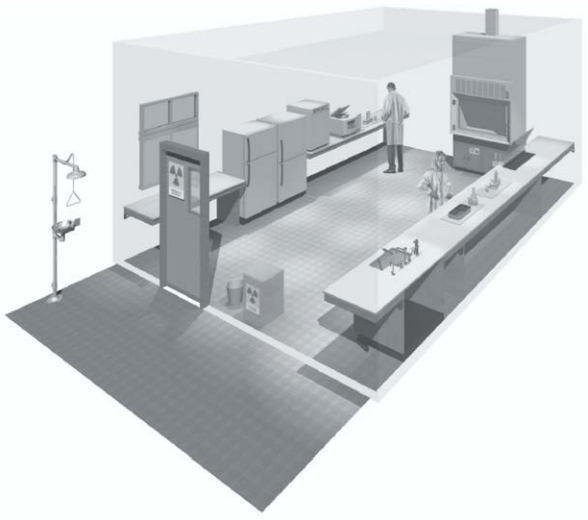


Figure 3. Typical BSL-2laboratory

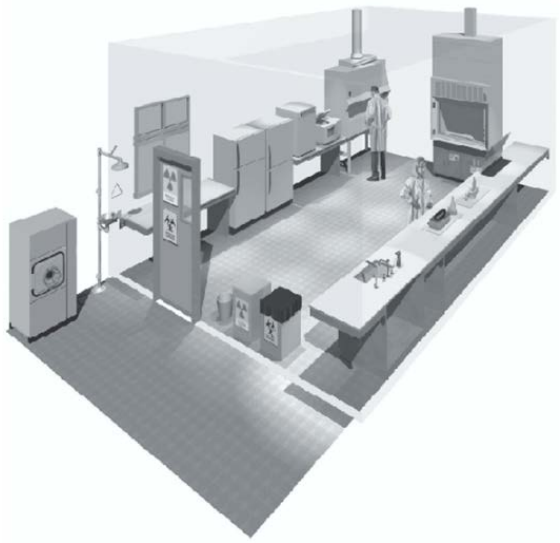


Figure 3. Biosafety Level 2 laboratory procedures likely to generate aerosols are performed within a biosafety cabinet. Doors are kept closed and are posted with appropriate hazard signs. Potentially contaminated wastes are separated from the general waste stream. Chemical fume hoods may not be required in all laboratories.(Graphics kindly provided by CUH2A, Princeton, NJ, USA)

1. Ample space must be provided for the safe conduct of laboratory work and for cleaning and maintenance.
2. Walls, ceilings and floors shall be smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory.
3. Floors shall be slip-resistant.
4. Bench tops shall be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.
5. Illumination shall be adequate for all activities. Undesirable reflections and glare should be avoided.
6. Laboratory furniture shall be sturdy, impervious to water and resistant to chemicals. Open spaces between and under benches, cabinets and equipment shall be accessible for cleaning.
7. Storage space near bench tops and aisles must be adequate to contain supplies for immediate use. Additional long-term storage space conveniently located outside the laboratory work areas shall be provided.
8. Space and facilities shall be provided for the safe handling and storage of solvents, radioactive materials and compressed or liquefied gases.
9. Storage facilities for outside wear garments and personal items shall be provided outside the laboratory work areas.
10. Facilities for eating, drinking and relaxation shall be available outside the laboratory work areas.
11. Hand-washing sinks,with running water if possible, shall be provided in each laboratory, preferably near the laboratory exit door.

12. Doors shall have vision panels, appropriate fire ratings, be self-closing and lockable.
13. Biosafety Level 2 facilities shall have doors that open into the laboratory and have an autoclave or other means of decontamination in appropriate proximity to the laboratory.
14. Safety equipment, including eyewash stations, fire suppression systems, electrical breakers and gas shut off valves should be in the laboratory work area. Emergency shower equipment shall be in appropriate proximity to the laboratory.
15. Firstaid equipment and supplies shall be readily accessible (see Annex 1).
16. Mechanical ventilation systems should provide directional inward flow of air into laboratory work areas without recirculation. If there is no mechanical ventilation, windows fitted with arthropod-proof screens may be opened.
17. A dependable supply of high quality research grade water should be provided. There shall be no cross-connection between research grade laboratory water and potable drinking-water supplies. Potable water systems shall be protected by backflow prevention devices.
18. Emergency lighting systems shall be installed in laboratory work areas. An emergency generator is desirable for the support of essential equipment, such as incubators, biosafety cabinets, freezers, etc. and for ventilation of animal cages.
19. Gas supply systems should have easily accessible gas shut off valves in laboratory work areas.
20. Laboratories and animal rooms are occasional targets of vandals. Physical and fire security equipment shall be present. Strong doors, screened windows and restricted issue of keys or cards shall be specified. Other measures may be considered, as appropriate, to augment security (see Chapter 8).
21. Glassware and other breakable materials shall be avoided whenever possible.
22. Procedures likely to generate aerosols should be performed within a biosafety cabinet or similar primary containment device.
23. Potentially contaminated wastes are separated from the general waste stream.

Laboratory equipment

Together with good procedures and practices, the use of safety equipment will help to reduce risks when dealing with biohazards. This section deals with basic principles related to equipment suitable for laboratories at all biosafety levels.

The laboratory director shall, after consultation with the biosafety officer and safety committee, ensure that adequate containment equipment is provided and laboratory personnel are trained to use the equipment.

Laboratory equipment shallcomply with international safety criteria. Equipment shall be:

1. Designed to prevent or limit contact between the operator and the infectious material.
2. Constructed of materials that are impermeable to liquids, resistant to corrosion and meet structural requirements.
3. Fabricated to be free of burrs, sharp edges and unguarded moving parts.
4. Designed, constructed and installed to facilitate simple operation and provide for ease of maintenance, cleaning, decontamination and certification testing.

Essential biosafety equipment

1. Pipetting aids shall be used; mouth pipetting is not permitted. Many different designs are available.
2. Biosafety cabinets (BSCs) or equivalent primary containment equipment shall be used whenever:
    a. Infectious materials are handled; such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biosafety cabinet.
    b. There is an increased risk of airborne transmission of infectious materials.
    c. Procedures that involve high kinetic energy that may produce aerosolsare used, such as centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressure may be different from ambient pressure, intranasal inoculation of animals and harvesting tissues from animals or eggs.
3. Plastic disposable transfer loops should be used to transfer microbial colonies.Alternatively, electric ovens may be used inside biosafety cabinets to sterilize wire transfer loops.
4. Screw-capped tubes and bottles should be used.
5. Autoclaves or other appropriate means to decontaminate infectious materials shall be used.
6. Plastic disposable Pasteur pipettes and other pipettes are used in place of glass when available.
7. Equipment such as autoclaves and biosafety cabinets must be certified or tested by appropriate methods before use. Recertification shall be performed annually or according to the manufacturer’s instructions and appropriate documents kept (see Chapter 7).

Health and medical surveillance

The biosafety committee is responsible for ensuring adequate health surveillance of laboratory personnelfor potential occupationally acquired diseases. This objective is accomplished by:

1. Provision of active or passive immunization where indicated (see Annex 2).

2. Facilitation of early detection of laboratory-acquired infections.
3. Exclusion of highly susceptible individuals (e.g. pregnant women or immune-compromised individuals) from highly hazardous laboratory work.
4. Provision of safe laboratory facilities, equipment, training and procedures.

Guidelines for surveillance of laboratory workers handling microorganisms at Biosafety Level 1

1. Microorganisms handled at Biosafety Level 1 are unlikely to cause human or animal disease. However, all laboratory workers shall undergo a recorded pre-employment health check and medical history.
2. All staff members shall promptly report illnesses or laboratory accidents.
3. All staff members shall receive good microbiological technique training.

Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 2

1. A recorded pre-employment or pre-placement occupational health assessment and medical history is required.
2. Records of illness and absence from work shall be kept by laboratory management.
3. Women of childbearing age shall be made aware of potential occupational exposure risks to an unborn child and what mitigation measures are available if certain microorganisms are used, e.g. rubella virus.

Training

Human error and poor technique can compromise safeguards designed to protect laboratory workers. A safety-conscious staff, well informed about the recognition and control of laboratory hazards, is key to the prevention of laboratoryacquired infections, incidents and accidents.

For this reason, continuous in-service safety training is essential. An effective safety program requires that laboratory managersensure that safe laboratory practices and procedures are integrated into employee basic training. Safety training shall be an integral part of new employees' introduction to laboratory research.

Training shall include the code of practice and local guidelines, including the safety or operations manual. Measures to assure that employees have read and understood the guidelines, such as signature pages, shall be adopted.

Laboratory supervisors provide a key training role for their immediate staff. The biosafety officer can assist with training and development of training aids and documentation (see also Chapter 20).

Staff training shall include safe methods for working with potentially hazardous procedures commonly encountered by most laboratory personnel, such as:

1. Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
2. Ingestion risks when handling specimens, smears and cultures.
3. Risks of percutaneous exposures when using syringes and needles.
4. Bites and scratches when handling animals.
5. Handling blood and other potentially hazardous pathological materials.
6. Decontamination and disposal of infectious material.

Waste handling

Waste is anything that is to be discarded.

Decontamination of laboratory waste and disposal are closely interrelated. In terms of daily use, few if any contaminated materials will require actual removal from the laboratory or destruction. Most glassware, equipment, instruments and laboratory clothing that has come into contact with potentially biohazardous material will be reused or recycled. The overriding principle is that all infectious materials shall be decontaminated by chemical or heat methods within the laboratory area.

Questions to ask before removing any potentially infectious objects, materials or animal tissuesare:

1. Have the objects or materials been effectively decontaminated or disinfected by an approved procedure?
2. If not, have they been packaged in an approved manner for immediate on-site incineration or transfer to another facility with incineration capacity?
3. Does the disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to those perform the immediate disposal procedures or who might come into contact with discarded items outside the facility?

Decontamination

1. Steam autoclaving is the preferred method for all decontamination processes.

2. Materials for decontamination and disposal shall be placed in containers, e.g. autoclavable plastic bags, which are color-coded according to whether the contents are to be autoclaved and/or incinerated.
3. Alternative methods may be used if they inactivate and/or kill microorganisms (for more details see Chapter 13).

Handling and disposal procedures for contaminated materials and wastes

An identification and separation system for biohazardous materials and containers shall follow national and international regulations. Categories may include:

1. Non-contaminated (non-infectious) waste that can be reused or recycled or discarded as general or municipal waste.
2. Sharps – hypodermic needles, scalpels, knives, pipette tips and broken glass; etc.shall be collected in sharps disposal containers designed for that purpose.
3. Contaminated material to be decontaminated by autoclaving followed by washing and reuse or recycling.
4. Contaminated material for autoclaving and disposal.
5. Contaminated material for direct incineration.

Sharps

After use, hypodermic needles shall not be recapped, clipped or removed from disposable syringes. The complete assembly shall be placed in a sharps disposal container. Disposable syringes, used alone or with needles, shall be placed in sharps disposal containers and incinerated without prior autoclaving,unless specially required.

Sharps disposal containers must have a biohazard label (Figure 1), be puncture-resistant on the sides and bottom and must not be filled to capacity. When they are three-quarters full,discard them into “infectious waste” containers for incineration without prior autoclaving,unless laboratory practice requires autoclaving before discarding. Sharps disposal containers must not be discarded into landfills.

Contaminated (potentially infectious) materials for autoclaving and reuse

No pre-cleaning should be attempted with any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

Contaminated (potentially infectious) materials for disposal

Except for sharps (refer to sharps section above), all contaminated (potentially infectious) materials shall be autoclaved in leak-proof containers, e.g. color-coded plastic autoclave bags, before disposal.

After autoclaving, solid materials may be placed in transfer containers for transport to an incinerator. When possible, materials from healthcare activities shall not be discarded in landfills even after decontamination. If a medical waste incinerator is available on site, autoclaving may be omitted and the contaminated waste shall be placed in designated containers (e.g. boxes or transfer carts) and transported directly to the incinerator.

Reusable transfer containers shall be leak proof and have tight-fitting covers. They shall be disinfected and cleaned before they are returned to the laboratory.

Discard containers, such as unbreakable plastic pans, beakersand/or small autoclave bags shall be placed at every work station. When disinfectants are used, waste materials should remain in direct contact with the disinfectant (i.e. not separated from the disinfectant by air bubbles) for an appropriate contact time, according to the disinfectant used (see Chapter 13). Plastic discard pans and beakers shall be decontaminated and washed before reuse.

Incineration procedures for contaminated waste must be approved by local public health and air pollution authorities, as well as the laboratory biosafety officer (see section on Incineration in Chapter 13).

Chemical, fire, electrical, radiation and equipment safety

Loss of biohazardous materialcontainment may result from chemical, fire, electrical or radiation accidentsin a microbiological laboratory. Statutory rules and regulations for chemical, fire, electrical and radiation safety are specified by national or local authorities.Their assistance with safety procedures should request. Chemical, fire, electrical and radiation hazards are considered in greater detail in Part VI of this manual (Chapters 16 and 17).Additional information regarding safety equipment is presented in Chapter 11.

3. High Containment Laboratory –Biosafety Level 3

The high containment Biosafety Level 3 laboratory is designed for work with Risk Group 3 microorganisms and large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread. Biosafety Level 3 containment requires increased operational and safety procedures beyond those for basic Biosafety Level 1 and containment Biosafety Level 2 laboratories.

The guidelines given in this chapter are in addition to those described above for basic and containment laboratories. Biosafety Level 3 laboratories are designed to be gas tight. The major changes for high containment laboratories are listed in the following sections:

1. Code of practice.
2. Laboratory design and facilities.
3. Health and medical surveillance.

Code of practice

The codes of practice for basic and containment laboratories, Biosafety Level 1 and Biosafety Level 2, respectively, are modified as follows:

1. The international biohazard warning symbol and sign (see Figure 1) displayed on laboratory access doors must identify the biosafety level, the name and emergency contact information for the laboratory supervisor who controls access, emergency contact information for the safety office and indicate any special conditions for entry into the area, e.g. immunization.
2. Laboratory protective clothing must be solid-front or wrap-around gowns, scrub suits or coveralls with gathered cuffs and when appropriate, head covering, shoe covers or dedicated shoes, safety eyewear, face mask or respiratory protection. Front-buttoned standard laboratory coats are not permitted and sleeves must fully cover the forearms. Laboratory protective clothing must not be worn outside the laboratory and must be decontaminated before it is laundered by the institution. Changing from street clothing into dedicated laboratory clothing may be warranted when working with certain agents (e.g. agricultural or zoonotic agents).
3. Manipulations of all potentially biohazardous materials must be performed within biosafety cabinets or other primary containment devices (see Chapter 10).
4. Respiratory protective equipment may be necessary for some laboratory procedures or working with animals infected with certain pathogens.

5. Face masks or surgical masks are recommended when working with all laboratory animals.

Laboratory design and facilities

The laboratory design and facilities for basic and containment laboratories apply except where modified as follows:

1. The laboratory must be separated from areas open to unrestricted traffic flow within the building. Separation is usually achieved by designing access through an anteroom (e.g. a double-door entry). The anteroom shall have areas for storage of clean supplies that are separated from used clothing and equipment discard areas. A shower may also be necessary for some research procedures.
2. Anteroom doors shall be self-closing and interlocking so that only one door can be opened at a time. A break-through panel, door breaker baror mechanical latch opening button may be provided for emergency exit use.
3. Surfaces of walls, floors and ceilings shall be water-resistant and easy to clean.
4. Horizontal wall penetrations and ceiling penetrations (no floor penetrations allowed) for service pipesshall be sealed to facilitate gaseous space decontamination of the room(s).
5. Floors shall be covered with integral cove, seamless vinyl.
6. Supply and exhaust ducting systems must be constructed so that they can be sealed for gaseous decontamination.
7. Windows must be closed, sealed and break-resistant.
8. A labeled hand-washing station with hands-free controls shall be provided near each exit door.
9. The independentlycontrolled ventilation system shall maintain directional airflow into the laboratory from the anteroom and from the adjoining public area into the anteroom. A visual monitoring device with or without alarm(s) shall be installed so that staff can at all times ensure that proper directional airflow into the laboratory room is maintained.
10. A dedicated building ventilation system for the laboratory must be constructed that is separate from other ventilation systems in the building. Air exhausted from the high containment laboratory is usually filtered through a pre-filter and a high-efficiency particulate air (HEPA) filter beforedischarge to the outside of the buildingaway from occupied buildings and air intakes. A heating, ventilation and air-conditioning (HVAC) control system must be installed to prevent positive pressurization of the laboratory, optimally shutting off air supply within thirty seconds after amechanical exhaust fan failure. Consideration should be given to the installation of audible and/or clearly visible alarms to notify personnel of HVAC system failure.
11. All pre-filters and HEPA filters must be installed in a manner that permits in-place gas decontamination and leak testing.

12. Biosafety cabinets shall be sited away from walking areas and out of crosscurrents from doors and ventilation systems. The preferred location is at the wall of the laboratory furthest from and perpendicular to the door (see Chapter 9).
13. The HEPA-filtered exhaust air from biosafety cabinets must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system.Classes II, Type A2 BSCs with a canopy connection to the laboratory mechanical exhaust system are recommended.
14. An autoclave for the decontamination of contaminated waste material should be available in the high containment laboratory. If infectious waste must be removed from the high containment laboratory for decontamination and disposal, it must be transported in sealed, unbreakable and leak proof containers according to national or international regulations, as appropriate.
15. Backflow-prevention devices must be fitted to the potable water supply. Vacuum lines should be protected with a liquid disinfectant trap with an aerosol filter between the disinfectant trap and the vacuum pump.Vacuum pumps should be located within the laboratory and be properly protected with traps and filters.
16. High containment laboratory – Biosafety Level 3 facility design and operational procedures shall be documented.

Figure 4. An example of laboratory design for Biosafety Level 3

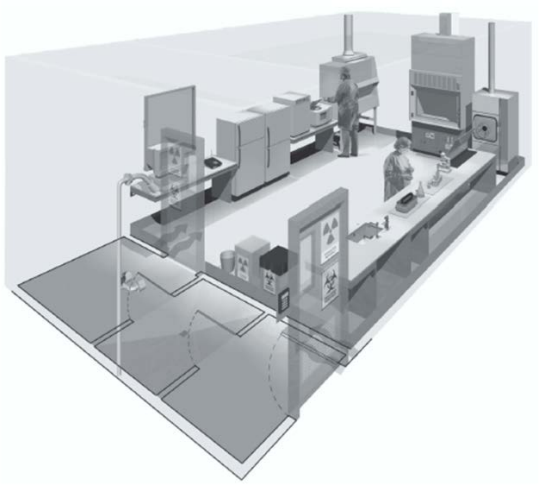


Figure 4. A typical high containment Biosafety Level 3 laboratory. The laboratory is separated from general traffic flow and accessed through an anteroom (double door entry). An autoclave is available within the facility for decontamination of wastes prior to disposal. A handwashing sink with hands-free operation is available near the exit. Inward directional airflow is established. All work with infectious materials is conducted within a biosafety cabinet or other suitable primary containment device.(Graphics kindly provided by CUH2A, Princeton, NJ, USA)

Laboratory equipment

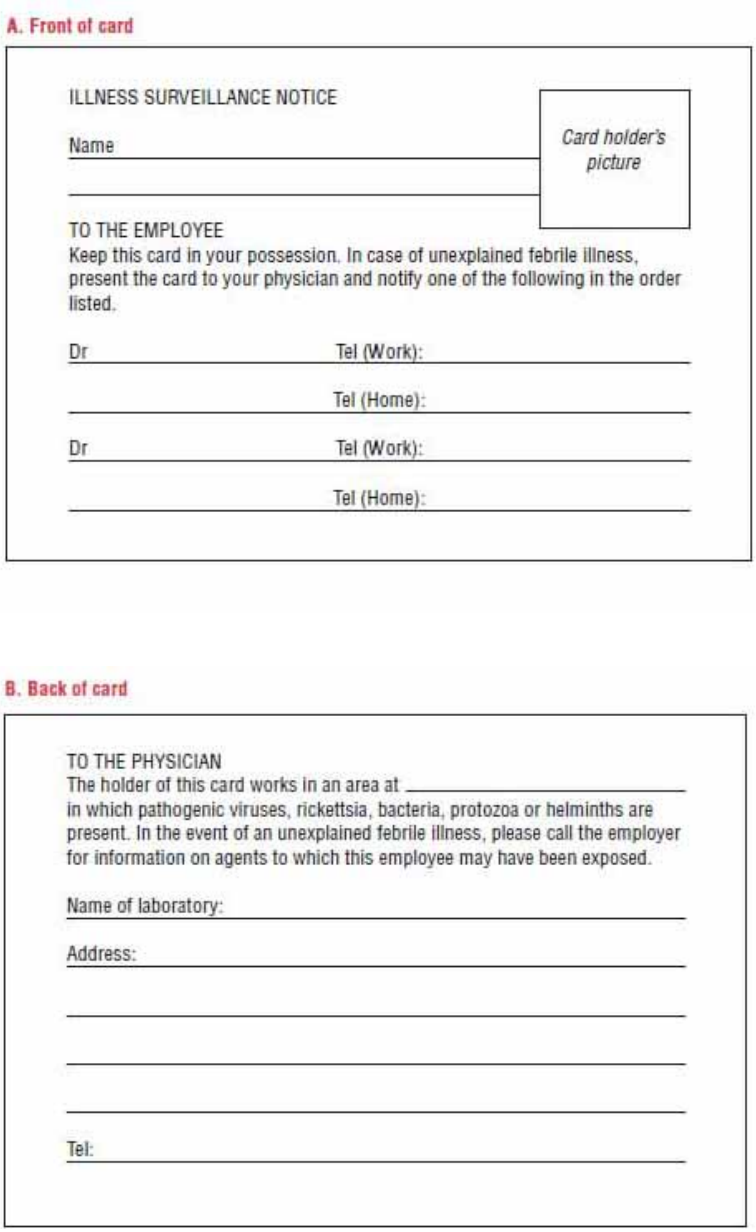
1. The principles for the selection of laboratory equipment, including biosafety cabinets, are the same as for the containment laboratory – Biosafety Level 2.
2. However, at Biosafety Level 3, manipulation of all potentially infectious material must be conducted within a biosafety cabinet or other primary containment device.
3. Consideration should be given to equipment such as centrifuges, which will need additional containment accessories, for example, safety buckets or containment rotors.
4. Some centrifuges and other equipment, such as cell-sorting instruments for use with infected cells, may need additional local exhaust ventilation with HEPA filtration for efficient containment.

Health and medical surveillance

The objectives of health and medical surveillance programs for basic laboratories (Biosafety Level 1) and containment laboratories (Biosafety Level 2) also apply to high containment laboratories (Biosafety Level 3) except where modified as follows:

1. Medical examination of all laboratory personnel who work in high containment Biosafety Level 3 laboratories is mandatory. This should include a recorded, detailed medical history and an occupationally-targeted physical examination.
2. After a satisfactory clinical assessment, the examinee may be provided with a medical contact card (e.g. as shown in Figure 5) stating that he or she is employed in a facility with a Biosafety Level 3 high containment laboratory. This card should include a picture of the card holder, be wallet-sized and always be carried by the holder.

Figure 5. Suggested format for medical contact card



A. Front of card

ILLNESS SURVEILLANCE NOTICE

Name _____

Card holder's picture

TO THE EMPLOYEE
Keep this card in your possession. In case of unexplained febrile illness, present the card to your physician and notify one of the following in the order listed.

Dr _____ Tel (Work): _____

Tel (Home): _____

Dr _____ Tel (Work): _____

Tel (Home): _____

B. Back of card

TO THE PHYSICIAN
The holder of this card works in an area at _____ in which pathogenic viruses, rickettsia, bacteria, protozoa or helminths are present. In the event of an unexplained febrile illness, please call the employer for information on agents to which this employee may have been exposed.

Name of laboratory: _____

Address: _____

_____

_____

_____

Tel: _____

4. Maximum Containment Laboratory – Biosafety Level 4

The maximum containment laboratory – Biosafety Level 4 is designed for work with Risk Group 4 microorganisms. Before such a laboratory is constructed and put into operation, intensive consultations must be held with institutions that have had experience operating a similar facility. The Ministry of Public Health will have an active role in the planning, design, construction and operation of the facility.

Because of the great complexity of the work in a Biosafety Level 4 laboratory, active cooperation with national and local health authorities shall be established. Other emergency services, e.g. fire, police and designated receiving hospitals, shall also be involved.

Operations of maximum containment laboratories shall be under the control of MOPH. The following information is intended only as introductory material. Entities considering development of a maximum containment Biosafety Level 4 laboratory must contact MOPH for additional information.

Code of practice

The code of practice for Biosafety Level 3 applies except where modified as follows:

1. The two-person rule shall apply, whereby no individual ever works alone. This is particularly important if working in a Biosafety Level 4 suit facility.
2. A complete change of clothing and shoes is required prior to entering and upon exiting the laboratory.
3. Personnel must be trained to perform emergency extraction procedures in the event of personnel injury or illness.
4. A method of communication for routine and emergency contacts must be established between personnel working within the maximum containment laboratoryand support personnel outside the laboratory.

Laboratory design and facilities

The features of a high containment laboratory (Biosafety Level 3) apply to a maximum containment laboratory (Biosafety Level 4) with addition of the following features.

1. Primary containment. An efficient primary containment system must be in place, consisting of one or a combination of the following:

> ***Class III cabinet laboratory.*** *Passage* through a minimum of two doors prior to entering the rooms containing the Class III biosafety cabinet(s) (cabinet room) is required. In this laboratory configuration, the Class III biosafety cabinet line provides the primary containment. A personnel shower with inner and outer changing rooms is necessary. Supplies and materials that are not brought into the cabinet room through the changing area are introduced through a double-door autoclave, dunk tank or gas decontamination chamber. Once the outer door is securely closed, staff inside the laboratory can open the inner door to retrieve the materials. The doors of the autoclave, dunk tank or gas decontamination chamber are interlocked in such a way that the outer door cannot open unless the autoclave has been operated through a sterilization cycle or the gas decontamination chamber has been decontaminated.
>
> ***Suit laboratory****.* A protective suit laboratory with positively pressurized, HEPA-filtered, supplied-air suitsdiffers significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biosafety cabinets. The rooms in the protective suit laboratory are arranged to direct personnel through changing and decontamination areas prior to entering areas where infectious materials are manipulated. A suit decontamination shower must be provided and used by personnel leaving the maximum containment laboratory area. A separate personnel shower with inner and outer changing rooms is also provided. Personnel who enter the suit area are required to don a one-piece, positively pressurized, HEPA-filtered, supplied-air suit. Air to the suit must be provided by a system that has a 100% redundant capability with an independent source of air for emergency use. Entry into the suit laboratory is through an airlock fitted with airtight doors. An appropriate warning system for personnel working in the suit laboratory must be provided for use in the event of mechanical system or air failure (see Chapter 10).

2. Controlled access*.* The maximum containment laboratory (Biosafety Level 4) must be located in a separate building or in a clearly delineated zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. Upon entering, personnel must put on a complete change of clothing. Before leaving, they must shower before putting on their street clothing.

3. Controlled air system.Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences between the ventilation systems of the Class III cabinet laboratory and suit laboratory:

> ***Class III cabinet laboratory****.* The supply air to the Class III biosafety cabinet(s) may be drawn from within the room through a HEPA filter mounted on the cabinet

or supplied directly through the supply air system. Exhaust air from the Class III biosafety cabinet(s) must pass through two HEPA filters prior to release outdoors. The cabinet must be operated at negative pressure to the surrounding laboratory at all times. A dedicated non-recirculating ventilation system is required.

Suit laboratory. Dedicated room HEPA-filtered air supply and exhaust systems are required. The supply and exhaust components of the ventilation system are balanced to provide directional airflow within the suit area from the area of least hazard to the area(s) of greatest potential hazard. Redundant exhaust fans are required to ensure that the facility remains under negative pressure at all times. The differential pressures within the suit laboratory and between the suit laboratory and adjacent areas must be monitored. Airflow in the supply and exhaust components of the ventilation system must be monitored and a system of controls must be used to prevent pressurization of the suit laboratory. HEPA-filtered supply air must be provided for the suit area, decontamination shower and decontamination airlocks or chambers. Exhaust air from the suit laboratory must be passed through a series of two HEPA filters prior to release outdoors. Alternatively, after double HEPA filtration, exhaust air may be recirculated, but only within the suit laboratory. Under no circumstances shall the exhaust air from the Biosafety Level 4 suit laboratory be recirculated to other areas. Extreme caution must be exercised if recirculation of air within the suit laboratory is elected. Consideration must be given to the types of research conducted, equipment, chemicals and other materials used in the suit laboratory, as well as animal species that may be involved in the research.

All HEPA filters need to be tested and certified every six months. The HEPA filter housings must be designed to allow for in situ decontamination of the filter prior to removal. Alternatively, the filter can be removed in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration.

4. Decontamination of effluents. All effluents from the suit area, decontamination chamber, decontamination shower or Class III biosafety cabinet must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment.

5. Sterilization of waste and materials.A double-door, pass-through autoclave must be located in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization.

6. Airlock entry ports for specimens, materials and animals must be provided.

7. Emergency powerand dedicated power supply line(s) must be provided.

8. Containment drain(s) must be installed.

5. Laboratory animal facilities

Those who use animals for experimental and diagnostic purposes have a moral obligation to take every care to avoid causing unnecessary pain or suffering. The animals must be provided with comfortable, hygienic housing and adequate wholesome food and water. At the end of the experiment they must be dealt with in a humane manner.

For security reasons, animal facilities shall be an independent, detached unit separated from laboratories and public areas. Animal facilities next to laboratories shall be isolated from public areas of the laboratory and capable of being decontaminated or disinfected.

Table 5 Animal facility containment levels: summary of practices and safety Equipment

RISK GROUP	CONTAINMENT LEVEL	LABORATORY PRACTICES AND SAFETY EQUIPMENT
1.	ABSL-1	Limited access, protective clothing, face masks and gloves.
2.	ABSL-2	ABSL-1 practices plus: hazard warning signs. Class I or II BSCs for activities that produce aerosols. Decontamination of waste and cages before disposal or washing.
3.	ABSL-3	ABSL-2 practices plus: controlled access. BSCs and special protective clothing for all activities.
4.	ABSL-4	ABSL-3 plus: strictly limited access. Clothing change before entering. Class III BSCs or positive pressure suits. Shower on exit. Decontamination of all wastes before removal from facility.

ABSL - animal facility Biosafety Level, BSCs - biosafety cabinets

Animal facilities, similar to laboratories, may be designated according to a risk assessment that includes the risk group of the microorganisms and procedures to be performed. They are designated as Animal Biosafety Level (ABSL) 1, 2, 3 or 4.

Risk analysis of the microorganisms and hazardous materials to be used in the animal laboratory includes consideration of:

1. The normal route of transmission.

2. The volumes and concentrations to be used.
3. The route of inoculation.
4. Whether hazardous materials may be excreted or shed by the animals.

With respect to animals to be used in the animal laboratory, factors for consideration include:

1. Their aggressiveness and tendency to bite and scratch.
2. Their natural ecto- and endo-parasites.
3. The zoonotic diseases to which they are susceptible.
4. The possible dissemination of allergens.

As with laboratories, animal facility design features, equipment and precautions increase in stringency as the animal biosafety level increases. These are described below and summarized in Table 5 (above). These guidelines are additive, so that each higher level incorporates the standards of the lower levels

Animal facility – Animal Biosafety Level 1

This is suitable for the maintenance of most stock animals after quarantine (except for nonhuman primates - national authorities must be consulted) and for animals that are deliberately inoculated with Risk Group 1 agents. Good microbiological technique (GMT)is required. The animal facility director must establish policies, procedures and protocols for all operations, including approval of personnel access to the vivarium. An appropriate medical surveillance program for the staff must be instituted. A safety or operations manual must be prepared and adopted.

Animal facility – Animal Biosafety Level 2

This is suitable for work with animals that are deliberately inoculated with Risk Group2 microorganisms. The following safety precautions apply:

1. All the requirements for animal facilities – Animal Biosafety Level 1 must be met.
2. Biohazard warning signs (see Figure 1) should be posted on doors and other appropriate places.
3. The facility must be designed for easy cleaning and housekeeping.
4. Doors must open inwards and be self-closing.
5. Heating, ventilation and lighting must be adequate.
6. If mechanical ventilation is provided, the airflow must be inward. Exhaust air is discharged to the outside and should not be recirculated to any part of the building.
7. Access must be restricted to authorized persons.
8. No animals shall be admitted other than those for experimental use.

9. There shall be an arthropod and rodent control program.
10. Windows, if present, must be secure, resistant to breakage and, if able to be opened, must be fitted with arthropod-proof screens.
11. After use, work surfaces must be decontaminated with effective disinfectants (see Chapter 13).
12. Biosafety cabinets (Class I or II) or isolator cages with dedicated air supplies and HEPA-filtered exhaust air must be provided for work that may involve the generation of aerosols.
13. An autoclave must be available on site or in appropriate proximity to the animal facility.
14. Animal bedding materials must be removed in a manner that minimizes the generation of aerosols and dust.
15. All waste materials and bedding must be decontaminated before disposal.
16. Use of sharp instruments should be restricted whenever possible. Sharps should always be collected in puncture-proof/-resistant containers fitted with covers and treated as infectious.
17. Material for autoclaving or incineration must be transported safely in closed containers.
18. Animal cages must be decontaminated after use.
19. Animal carcasses should be double-bagged and refrigerated before transport to a pathologic incinerator.
20. Protective clothing and equipment must be worn in the facility and removed before leaving. All personnel must wear face masks in the facility.
21. All protective clothing must be decontaminated before it is laundered.
22. Hand washing facilities supplied with running water and mild, non-antimicrobial soap must be provided. Staff must wash their hands before leaving the animal facility.
23. All injuries, however minor, must be treated appropriately, reported and recorded.
24. Eating, drinking, chewing, smoking, handling contact lenses and application of cosmetics are forbidden in the facility.
25. All personnel must receive appropriate training.

Animal facility – Animal Biosafety Level 3

This is suitable for work with animals that are deliberately inoculated with Risk Group 3 agents or when otherwise indicated by a risk assessment. All systems, practices and procedures need to be reviewed and recertified annually. The following safety precautions apply:

1. All the requirements for animal facilities – Animal Biosafety Levels 1 and 2 must be met.
2. Access must be strictly controlled.

3. The facility must be separated from other laboratory and animal facilities by ananteroom with an interlocked double-door entrance.
4. Hand washing facilities supplied with running water and mild, non-antimicrobial soap must be provided in the anteroom.
5. Showers shall be provided in the anteroom.
6. There must be mechanical ventilation to ensure a continuous airflow through all the rooms. Exhaust air must pass through HEPA filters before being discharged to the atmosphere without recirculation. The system must be designed to prevent accidental reverse flow or positive pressurization in any part of the animal facility.
7. An autoclave must be available at a location convenient to the animal facility where biohazardsare contained. Infectious waste should be autoclaved before it is removed and moved to other areas.
8. A pathologic waste incinerator shall be readily available on site or alternative arrangements should be made with the appropriate authorities.
9. Animals infected with Risk Group 3 microorganisms must be housed in negative air pressure cage racks of isolator cages.
10. Bedding should be as dust-free as possible.
11. All protective clothing must be decontaminated before it is laundered.
12. Windows must be shatter resistant, closed and sealed.
13. The rooms shall be capable of being sealed for gas decontamination.
14. Immunization of staff shall be offered, as appropriate.

Animal facility – Animal Biosafety Level 4

Work in this facility will normally be linked with a maximum containment laboratory – Biosafety Level 4.MOPHrules and regulations apply to both. If work is to be done in a suit laboratory, additional practices and procedures must be used over and above those described here (see Chapter 5).

1. All the requirements for animal facilities – Animal Biosafety Levels 1, 2 and 3 must be met.
2. Access must be strictly controlled; only staff designated by the facility director shall be authorized to enter the facility.
3. Individuals must not work alone: the two-person rule must apply.
4. Personnel must have the highest possible level of training as microbiologists and be familiar with the hazards involved in their work and necessary precautions.
5. Housing areas for animals infected with Risk Group 4 agents must maintain the criteria for containment described and applied for maximum containment laboratories – Biosafety Level 4.
6. The facility must be entered by an airlock anteroom, the clean side of which must be separated from the restricted side by changing and showering facilities.

7. Staff must remove street clothing when entering and put on protective clothing. After work they must shower and remove the protective clothing for autoclavingbefore leaving.
8. The facility must be ventilated by a dedicated, redundant HEPA-filtered supply and exhaust system designed to ensure a negative pressure (inward directional airflow).
9. The ventilation system must be designed to prevent reverse flow or positive pressurization.
10. A double-ended autoclave with a bioseal between the laboratory wall and the room outside containment must be provided for removal of decontaminated materials.
11. A pass-through airlock with the clean end in a room outside containment must be provided for exchange of non-autoclavable materials.
12. All manipulations with animals infected with Risk Group 4 agents must take place under maximum containment – Animal Biosafety Level 4 conditions.
13. All animals must be housed in isolators.
14. All animal bedding and waste must be treated (decontaminated) before removal from the facility.
15. The rooms shall be capable of being sealed for gas decontamination.
16. There must be medical supervision of staff.

Invertebrates

As with vertebrates, the animal facility biosafety level will be determined by the risk groups of the agents under investigation or when otherwise indicated by a risk assessment. The following additional precautions are necessary with certain arthropods, particularly flying insects:

1. Separate rooms should be provided for infected and non-infected invertebrates.
2. The rooms shall be capable of being sealed for gasdecontamination.
3. Insecticide sprays shall be readily available.
4. “Chilling” facilities shall be provided to reduce, where necessary, the activity of invertebrates.
5. Access shall be through an anteroom containing insect traps and arthropod-proof screens on the doors.
6. All exhaust ventilation ducts and openable windows should be fitted with arthropod-proof screens.
7. Waste traps on sinks and sluices must be kept filled with water/disinfectant.
8. All waste shall be decontaminated by autoclaving, because some invertebrates are not killed by all disinfectants.
9. A log shall be kept of the numbers of larval and adult forms of flying, crawling and jumping arthropods.
10. Containers for ticks and mites shall be placed in trays of oil or other suitable liquid.

11. Infected or potentially infected flying insects must be contained in double-netted cages.
12. Infected or potentially infected arthropods must be handled in biosafety cabinets or isolators.
13. Infected or potentially infected arthropods may be manipulated on cooling trays.

For further information, see references (3–6).

6. Guidelines for laboratory/facility commissioning

Laboratory/facility commissioning may be defined as the systematic review and documentation process that documents specified laboratory structural components and systems and/or system components have been installed, inspected, functionally tested and verified to meet national or international standards, as appropriate. The respective building system's design criteria and design function establish these commissioning requirements. In other words, laboratories designated as Biosafety Levels 1–4 will have different and increasingly complex commissioning requirements. Geographic and climatic conditionssuch as geological fault lines or extreme heat, cold or humidity may affect the laboratory design and therefore the commissioning requirements. Upon the completion of the commissioning process, the pertinent structural components and support systems will have been subjected to the various operating conditions and failure modes that can be reasonably expectedand will have been approved by the Ministry of Public Health.

The commissioning process and acceptance criteria shall be established early, preferably during the programming phase of the construction or renovation project. By acknowledging the commissioning process early in the project, architects, engineers, safety and health personnel and ultimately the laboratory occupants will understand the performance requirements of the specific laboratory. The commissioning process provides the institution and the surrounding community with confidence that the structural, electrical, mechanical, plumbing, containment, decontamination systems, security and alarm systems will operate as designed. This will assure containment of any potentially biohazardous microorganisms being worked with in a particular laboratory or animal facility.

Commissioning activities generally begin during the programming phase of the project and proceed through the construction and subsequent warranty period for the laboratory/facility. Warranty periods shall generally extend for one year following occupancy. It is recommended that a commissioning agent be retained who is independent of the architectural, engineering and construction firms involved in the design and construction. The commissioning agent serves as an advocate for the institution that owns the facility and shall be considered a member of the design team and shall be involved in the early programming phase of the project. In some cases, the institution may act as its own commissioning agent. For more complex laboratory facilities (Biosafety Levels 3 or 4), MOPH will have an active oversight role. The institution may wish to retain an outside commissioning agent who has demonstrated experience and success with commissioning of complex biosafety laboratory and animal facilities. When an independent commissioning agent is used,an institutional representative(s) shallalso be a member of

the commissioning team. It is recommended that, in addition to the commissioning agent, the Ministry of Public Health, the institution's Safety Officer, Project Officer, Program Manager, the principal investigator or research director and a representative of the Operations and Maintenance staff be part of the design team.

The following is a list of laboratory systems and components that may be included in a commissioning plan for functional testing. The systems and components may vary depending on the containment level of the facility being renovated or constructed. The list is not all-inclusive. The actual commissioning plan will reflect the complexity of the laboratory being planned:

1. Building automation systems including links to remote monitoring and control sites.
2. Electronic surveillance and detection systems.
3. Electronic security locks and proximity device readers.
4. Heating, ventilation (supply and exhaust) and air-conditioning (HVAC) systems.
5. High-efficiency particulate air (HEPA) or ultra-low-penetrating air (ULPA) filtration systems.
6. HEPA/ULPA decontamination systems.
7. HVAC and exhaust air system controls and control interlocks.
8. Airtight isolation dampers.
9. Laboratory refrigeration systems such as refrigerators, freezers and cold rooms.
10. Boilers and steam systems.
11. Fire detection, suppression and alarm systems.
12. Domestic water backflow prevention devices.
13. Processed water systems (i.e. reverse osmosis, distilled water).
14. Liquid effluent treatment and neutralization systems.
15. Plumbing drain primer systems.
16. Chemical decontamination systems.
17. Medical laboratory gas systems.
18. Breathing air systems.
19. Service and instrument air systems.
20. Cascading pressure differential verification of laboratories and support areas.
21. Local area network (LAN) and computer data systems.
22. Normal electrical power systems.
23. Emergency electrical power systems.
24. Uninterruptible power systems.
25. Emergency lighting systems.
26. Lighting fixture penetration seals.
27. Electrical and mechanical penetration seals.
28. Telephone and Wi-Fisystems.
29. Airlock or anteroom door control interlocks.
30. Airtight door seals.
31. Window and vision-panel penetration seals.

32. Barrier pass-through penetrations.
33. Structural integrity verification: concrete floors, walls and ceilings.
34. Barrier coating verification: floors, walls and ceilings.
35. Biosafety Level 4 containment envelope pressurization and isolation functions.
36. Biosafety cabinets.
37. Autoclaves.
38. Liquid nitrogen system and alarms.
39. Water detection systems (e.g. in case of flooding inside containment zone).
40. Decontamination shower and chemical additive systems.
41. Cage-wash and neutralization systems.
42. Waste management.

7. Guidelines for laboratory/facility certification

Laboratories are complex and dynamic environments. Today's biomedical research and clinical laboratories must be able to adapt quickly to continuously increasing public health needs and pressures. An example of this is the need for laboratories to adjust priorities to meet the challenges of emerging or re-emerging infectious diseases. In order to assure that adaptation and maintenance are undertaken promptly and in an appropriate and safe manner, all biological research and clinical laboratories should be regularly certified. Laboratory certification helps to ensure that:

1. Proper engineering controls and management systems are being used and are functioning adequately as designed.
2. Appropriate site and protocol specific administrative controls are in place.
3. Personal protective equipment is appropriate for the tasks being performed.
4. Decontamination of waste and materials has been adequately considered and proper waste management procedures are in place.
5. Proper procedures for general laboratory safety, including physical, electrical and chemical safety are in place.

Laboratory certification differs from laboratory commissioning activities (Chapter 6) in several important ways. Laboratory certification is the systematic examination of all safety features and processes within the laboratory (engineering controls, personal protective equipment and administrative controls). Biosafety practices and procedures are also examined. Laboratory certification is an on-going quality and safety assurance activity that should take place on a regular basis; at least annually.

MOPH trained safety and health or biosafety professionals may conduct laboratory certification activities. Institutions may employ personnel having the appropriate skill-sets required for conducting audits, surveys or inspections. However, institutions may consider engaging or be required to engage a third party to provide these services.

Biomedical research and clinical laboratory facilities may develop audit, survey or inspection tools to help ensure consistency in the certification process. These tools should be flexible enough to allow for the physical and procedural differences between laboratories necessitated by the type of work being conducted, while at the same time providing a consistent approach throughout the institution. Care must be taken to ensure that these tools are used only by appropriately trained personneland that they are not used as a substitute for a sound, professional biosafety assessment. Examples of such tools are provided in Tables 6–8.

Findings of the audit, survey or inspection shall be discussed with laboratory personnel and management. A biosafety committee shall be identified and made responsible for ensuring that corrective actions are taken for all deficiencies identified during the audit process. Certification of the laboratory shall not be completed and the laboratory shall not be declared functional until deficiencies have been adequately addressed and approved by the biosafety committee.

PART II

Laboratory biosecurity

8.Laboratory biosecurity concepts

The laboratory biosafety manual has in the past focused on traditional biosafety guidance for laboratories. The manual emphasizes the use of standard microbiological practices, appropriate containment equipment, proper facility design, operation and maintenance and administrative considerations to minimize the risk of worker injury or illness. By following these recommendations, the risk to the environment and surrounding community-at-large is also minimized. It has now become necessary to expand this traditional approach to biosafety by adding laboratory biosecurity measures. Global events in the recent past have highlighted the need to protect laboratories and the materials they contain from being intentionally compromised in ways that may harm people, livestock, agriculture or the environment. Before the laboratory biosecurity needs of a facility can be defined, however, it is important to understand the distinction between “laboratory biosafety” and “laboratory biosecurity”.

“Laboratory biosafety” is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional worker exposure to pathogens and toxins or their accidental release. “Laboratory biosecurity” refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

Effective biosafety practices are the foundation of laboratory biosecurity activities. Through risk assessments, performed as an integral part of an institution’s biosafety program, information is gathered regarding the type of organisms available, their physical location, the personnel who require access to them and the identification of those individuals responsible for them. This information can be used to assess whether an institution possesses biological materials that are attractive to those who may wish to use them improperly.

A specific laboratory biosecurity program must be prepared and implemented for each facility according to the requirements of the facility, the type of laboratory work conducted and the local political conditions.

Laboratory biosecurity measures should be based on a comprehensive program of accountability for pathogens and toxins that includes an updated inventory with storage location, identification of personnel with access, description of use, documentation of internal and external transfers within and between facilities and any inactivation and/or disposal of these materials. Likewise, an institutional laboratory biosecurity protocol shall be established for identifying, reporting, investigating and remediating breaches in laboratory biosecurity, including discrepancies in inventory results.

Laboratory biosecurity training distinct from laboratory biosafety training shall be provided to all personnel. Such training should help personnel understand the need for protection of such materials and the rationale for specific biosecurity measures. The training shall include a review of relevant MOPH standards and institution-specific procedures. Procedures describing the security roles and responsibilities by personnel in the event of a security infraction shall be presented.

The professional and ethical suitability of all personnel who have regular authorized access to sensitive materials and/or work with pathogens is central to an effective laboratory biosecurity program.

In summary, security precautions should become a routine part of laboratory work, including appropriate aseptic techniques and microbiological practices. Laboratory biosecurity measures should not hinder the efficient sharing of reference materials, clinical and epidemiological specimens or related information necessary for clinical or public health investigations. Competent security management should not unduly interfere with the day-to-day activities of scientific personnel or be an impediment to conducting research. Legitimate access to important research and clinical materials must be preserved. Assessments of the suitability of personnel, security-specific training and rigorous adherence to pathogen protection procedures are a reasonable means of enhancing laboratory biosecurity. All such efforts must be established and maintained through regular risk and threat assessments and regular review and updating of procedures. Checks for compliance with these procedures, with clear instructions of roles, responsibilities and remedial actions for personnel, should be integral to laboratory biosecurity programs and national standards for laboratory biosecurity.

PART III

Laboratory equipment

9. Biosafety cabinets

Class II and Class III Biosafety Cabinets (BSCs) are designed to protect the operator, the laboratory environment and work product from exposure to contaminants, infectious microorganisms and other biohazards. BSCs contain aerosols and splashes that may be generated when working with microbiological cultures, primary cell cultures, culture stocks, diagnostic specimens and chemotherapeutics. Aerosol particles are created by activities that impart high kinetic energy into liquids or semiliquid materials, such as shaking, pouring, stirring, transferring or dropping liquid onto surfaces or into other liquids. Transferring colonies to agar plates, inoculating cell culture flasks, using multichannel pipettes to dispense liquid suspensions into microculture plates, homogenizing, vortexing, centrifugation and working with animals can generate infectious aerosols.

Aerosol particles less than 5 µm in diameter and small droplets of 5–100 µm in diameter are not visible to the naked eye. Laboratory workersare generally not aware that such particles are being generated. These particles may be inhaled or may cross contaminate materials on the work surfaces. BSCs, when properly used, effectively reduce laboratory-acquired infections, cross-contamination of cultures and protect the laboratory and outside environment.

Over the years the basic design of BSCs has undergone several modifications. A major advancement came with the development of high-efficiency particulate air (HEPA) or ultra-low penetrating air (ULPA) filtersforBSC exhaust systems. HEPA filters remove at least 99.99% of airborne particle size ranges of 0.1 to 0.2 µm or 0.2 to 0.3 µm diameter. ULPA filters remove at least 99.999% of airborne particle size ranges of 0.1 to 0.2 µm or 0.2 to 0.3 µm diameter. The upgrade to ULPA filters can be specified at the time of cabinet purchase and is available as a standard option from suppliers. HEPA/ULPA filters are more efficient when removing particles smaller than or larger than 0.3 µm diameter. This enables HEPA/ULPA filters to effectively trap all known infectious agents and macromolecules, which ensures that only microbe-free exhaust air is discharged from the cabinet.

A second design modification was to direct HEPA/ULPA-filtered air over the work surface, providing protection of work surface materials from contamination. This feature is referred to as product protection. These basic design concepts have led to the evolution of three classes of BSCs. The type of protection provided by each is presented in Table 6.

One of the most difficult tasks when selecting a BSC is trying to foresee all of the different types of work that may take place in the BSC. It is critical to decide what things need protection, both now and in the future. All too often, users purchase a Unidirectional Flow

Clean-Air Device (IEST RP CC002, latest revision) (Clean Air Bench) or Class I BSC for current applications, only to find these devices are unsuitable as their work requirements change. Tables 6 and 7 list the characteristics of biosafety cabinets.

Note. Horizontal and vertical unidirectional flow clean-air devices ("clean-air benches or work stations") are not biosafety cabinets and should not be used in place of a biosafety cabinet.

Table 6. Selection of biosafety cabinet (BSC) by type of protection need

Type of Protection	BSC Selection
Personnel protection, Biosafety Levels 1-3	Class I
Personnel,product& environmentalprotection, Biosafety Levels1–3	Class II, Class III
Personnel, product & environmental protection, Biosafety Level 4, cabinet laboratory	Class III
Personnel, product & environmental protection, Biosafety Level 4, suit laboratory	Class II, Class III
Use of volatile chemical/radionuclides (a)	Class II canopy-connected Type A1 or A2; Type B1 or B2; Class III

(a) An independent chemical/radionuclide risk assessment should be performed by the investigator to determine safe amounts of chemicals/radionuclides permissible for their specific BSC installation; based on exhaust type, e.g. recirculating to the laboratory or connected to a mechanical exhaust system (canopy or solid connection) and the BSC's exhaust volume.

Table 7. Differences between Class I, II and III biosafety cabinets (BSCs)

BSC	(%) Air Exhausted	Exhaust System
Class I	100%	Direct duct or exhaust to lab
Class II Type A1 or A2	Varies by model	Exhaust to lab or Canopy connection
Class II Type B1	Greater than 50%	Direct duct
Class II Type B2	100%	Direct duct
Class III	100%	Direct duct

Selection of a biosafety cabinet

Selecting the proper BSC should be done in two stages:First, select the proper class and type of cabinet required; Second, decide on the size of the cabinet and options that are needed (5and 7-16). The various configurations of Class II BSCs are shown in figures 7, 8, 9 and 10. Deciding which Class and Type is appropriate can be accomplished by answering the following five questions.

1. What needs to be protected?

1. Protect only the material being worked on (product protection).
2. Protect only the technician and the laboratory (personnel and environmental protection).
3. Protect all three (personnel, product, and environmental protection).

If all that is needed is product protection, then a Unidirectional Flow Clean-Air Device, which is not a BSC, may be the unit of choice. Clean air devices use a High Efficiency Particulate Air (HEPA) or Ultra Low Penetration Air (ULPA) filter to remove particulates from room air. This filtered, particulate-free air then flows through an enclosed work area in a horizontal or vertical direction. These devices bathe the materials inside with filtered air and then the air is typically discharged into the laboratory. While these devices protect the product from airborne contaminants, any aerosol generated in the work area will be discharged into the laboratory and expose the operator. They cannot be used with volatile organic chemicals, chemotherapeutic agents, toxic or biohazardous materials.

For personnel and environmental protection only, the Class I biosafety cabinet may offer a simple and economical solution. Room air sweeps around the operator and across the work area. This contaminated air is then HEPA- or ULPA- filtered and discharged into the

laboratory or exhausted outside of the building via an external mechanical exhaust system. The Class I biosafety cabinet will protect the operator and the lab. However, because room air constantly washes over the work area, the product is exposed to airborne contaminants.

Personnel, environmental and product protection is most efficiently provided by a Class II biosafety cabinet. The inflow of air around the operator provides personnel protection. HEPA- or ULPA-filtered air flowing downward through the work area provides product protection and protects the laboratory from biohazardous particulates.

2. What are all of the different types of work to be done in the cabinet?

One of the most difficult tasks in selecting a BSC is trying to foresee all the different types of work that will be taking place in the BSC. It is critical to decide what things need protection, both now and in the future. All too often users purchase a clean-air device or Class I biosafety cabinet for work with clean or sterile materials or materials that do not need to be protected from contamination, only to find that these devices are unsuitable as their work requirements change to require personnel, environmental and product protection.

3. What types and quantities of chemical vapors will be generated in the BSC?

As important as the preceding question, the user must also foresee the types and quantities of chemical vapors that will be generated in the cabinet. Because chemical vapors can freely pass through HEPA or ULPA filters, both Class I and Class II BSCs must be exhausted out of the laboratory when used with these types of chemicals. Class II Type B1 and B2 biosafety cabinets must be direct ducted to an external exhaust system in order to operate properly. Class II Type A1 and A2 biosafety cabinets must operate in a canopy connected mode for work with significant quantities volatile chemicals.

An independent chemicalrisk assessment should be performed by the investigator to determine safe amounts of chemicals permissible for their specific BSC installation, based on evaluation of exhaust type, e.g. recirculating to the laboratory or direct connected to mechanical exhaust system (canopy or direct connection) and the BSC exhaust volume.

When flammable or explosive chemicals are to be used in a BSC, it is the users' responsibility to be fully cognizant with the properties of chemical(s) and the hazards associated with them:

1. Calculate the highest percent of recirculation that may occur in the BSC being used.

2. Ensure the concentration of chemical(s) released in the work area do not exceed their explosive limit.
3. Utilize the lowest quantities of the chemical(s) required for the procedure being performed.
4. Have appropriate spill/splash cleanup procedures in place before using the chemical(s).
5. The independent chemical risk assessment should determine safe amounts of chemicals permissible for their specific BSC installation based on the quantity(s) and name(s) of volatile chemical(s) and the BSC's air exhaust volume and exhaust configuration.

Possible BSC exhaust configurations are listed below:

1. Some Class I BSCs recirculate to the laboratory.
2. Class II Type A1 and Type A2 BSCs recirculate to the laboratory.
3. Class II Type A1 and Type A2 BSCs canopy connect to the laboratory mechanical exhaust system.
4. Some Class I and all Class II Type B1 and B2 BSCs direct duct to a dedicated mechanical exhaust system with independent ducting and exhaust fan for each BSC.

4. Is there an appropriate location for the cabinet ductwork?

If a Type A BSC is going to recirculate its HEPA- or ULPA-filtered air back into the laboratory, then the user has some freedom as to where the unit can be installed, provided it is out of major traffic areas and there are no other air handling devices in the area. Type B BSCs require a direct ducted, dedicated exhaust system with a dedicated exhaust fan located at or near the roof of the building for each BSC, so the location of the cabinet becomes dependent on the location of the dedicated exhaust system. The exhaust duct must be placed so it can penetrate ceilings and floors without disturbing other ventilation or plumbing systems. The exhaust system must also be designed to minimize excessive lengths and elbows. Direct ducting Type A cabinets is not allowed – they are exhausted through a canopy connection to the laboratory mechanical exhaust system.

BSCs not connected to an exhaust system should have at least 12 in (30 cm) clearance between the top of the BSC exhaust filter face and any overhead obstructions to allow for filter leak testing and velocity measurements of the exhaust HEPA/ULPA filter airflow with a thermal anemometer when used to calculate cabinet inflow velocity.

BSCs connected to an exhaust system should have at least 12 in (30 cm) clearance to allow for the passage of a 10 in (25 cm) or 12 in (30 cm) diameter duct and 100% shut off damper. Avoid cabinet locations that require either an elbow directly on top of the cabinet's exhaust connection or an excessive number of elbows to clear other items.

If a Type A BSC operates in recirculation mode, the HEPA/ULPA-filtered exhaust air will return to the laboratory. The BSC exhaust volume is not much, usually 300 cfm (0.14 m^3/s) greater than the laboratory ventilation requirement for air changes. The added cost of canopy connecting Type A BSCs is the cost of the exhaust canopy. No additional building

exhaust fans or ductwork is needed. The building mechanical exhaust should be connected with flexible duct over the BSC exhaust outlet and contain a manual damper to isolate the BSC for service or space decontamination. The canopy connection also removes heat generated by the BSC and functions as the laboratory exhaust outlet.

Type B BSCs (Type B1 or Type B2) are considerably more expensive to operate because they exhaust a greater air volume and require a greater exhaust duct static pressure. Type B BSCs shall have their own dedicated exhaust ductwork, rooftop bag-in-bag-out HEPA/ULPA filters and dedicated exhaust fan. The exhaust duct must be placed so it can penetrate ceilings and floors without disturbing other ventilation or plumbing systems. The exhaust system must also be designed to minimize excessive lengths and elbows.B2 BSCs are more complex and difficult to keep operating correctly. They should be avoided unless the microbiological procedures require significantly large quantities of volatile organic chemicals.

5. If the volume of air being removed by the BSC's exhaust system is reduced or eliminated, due to malfunction, what is its effect on BSC performance and what is preferred by the user?

For Type A BSCs fitted with a properly designed canopy connection, reduction or elimination of the exhaust air should not significantly affect the airflow patterns within the BSC. Personnel and product protection of the BSC will remain unchanged; however, chemical vapors generated in the BSC will be exhausted into the laboratory via the openings or slots in the exhaust canopy.

For Type B BSCs, any reduction or elimination of the exhaust air (such as BSC alarm) will directly impact the BSC’s inflow velocity and thus the personnel protection offered by the BSC. Loss of the exhaust airflow will eliminate the inflow of air into the front of the BSC, negating personnel, product and environmental protection. Airborne materials in the work area of the BSC will escape into the laboratory, thus creating a hazard to personnel.

Type B BSCs have operational and maintenance issues that must be considered:

1. These cabinets exhaust as much as 1200 cubic feet (34 cubic meters) per minute of conditioned room air, making them relatively expensive to operate.
2. The higher static air pressure required to operate Type B cabinets may also result in additional construction costs associated with heavier gauge ductwork and higher capacity exhaust fan.

Class I biosafety cabinet

Figure 6 provides a schematic diagram of a Class I BSC. A Class I BSC provides personnel and environmental protection without product protection. Personnel protection is provided bydrawing in laboratory air at a minimum velocity of 75 ft/min (0.38 m/s) through the front opening and across the work surface. The air is then passed through a HEPA/ULPA filter in the exhaust plenum, providing environmental protection. Some Class I BSCs exhaust into the laboratory.

The Class I BSC was the first recognized biosafety cabinet and because of its simple design, isstill used. It has the advantage of providing personneland environmental protection and can also be used for work with radionuclides andvolatile toxic chemicals if the exhaust is direct connected to the mechanical exhaust system. But there is no product protection because room air is drawn over the work surface through the front opening.

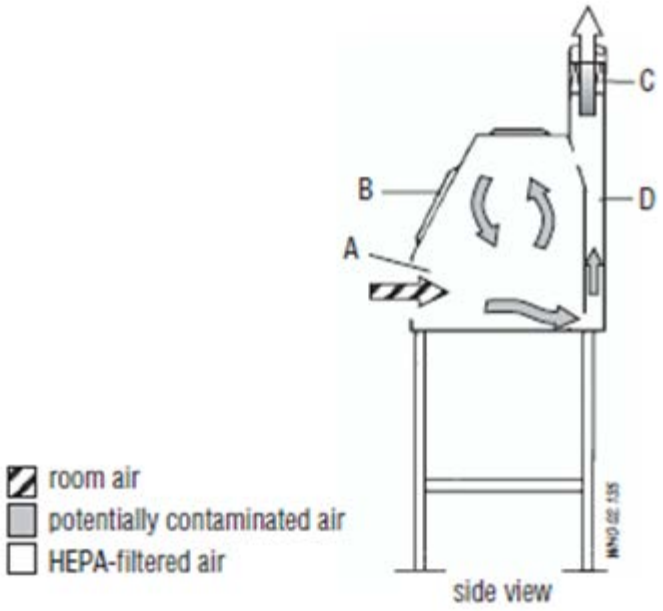


Figure 6. Schematic diagram of a Class I biosafety cabinet.
A, front opening; B, sash; C, exhaust HEPA filter; D, exhaust plenum.

Class II biosafety cabinet

As research involving cell and tissue culture for propagation of viruses and other studies became common, Class I BSC use decreased. Researchers did not want contaminated room air passingover their work surface and contaminating the material they were working with. Class II (Type A1, A2, B1 and B2) BSCs are partial barrier systems that rely on the

movement of air to provide personnel, environmental and product protection. Personnel and product protection is provided by the combination of inward and downward airflow captured by the front and rear grills of the cabinet.

Side-to-side cross-contamination of product is minimized by the internal downward flow of HEPA/ULPA filtered air moving towards the work surface into the front and rear intake grills. Environmental protection is provided when cabinet exhaust air is passed through a HEPA/ULPA filter. When used as designed, the cabinet exhaust air may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection (Type A1 and A2 BSCs). Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a sealed connection.

All Class II cabinets are designed for work involving procedures assigned to biosafety levels 1, 2 and 3. Class II BSCs may be used with procedures requiring BSL-4 containment if used in a BSL-4 suit laboratory by a worker wearing a positive pressure protective suit.

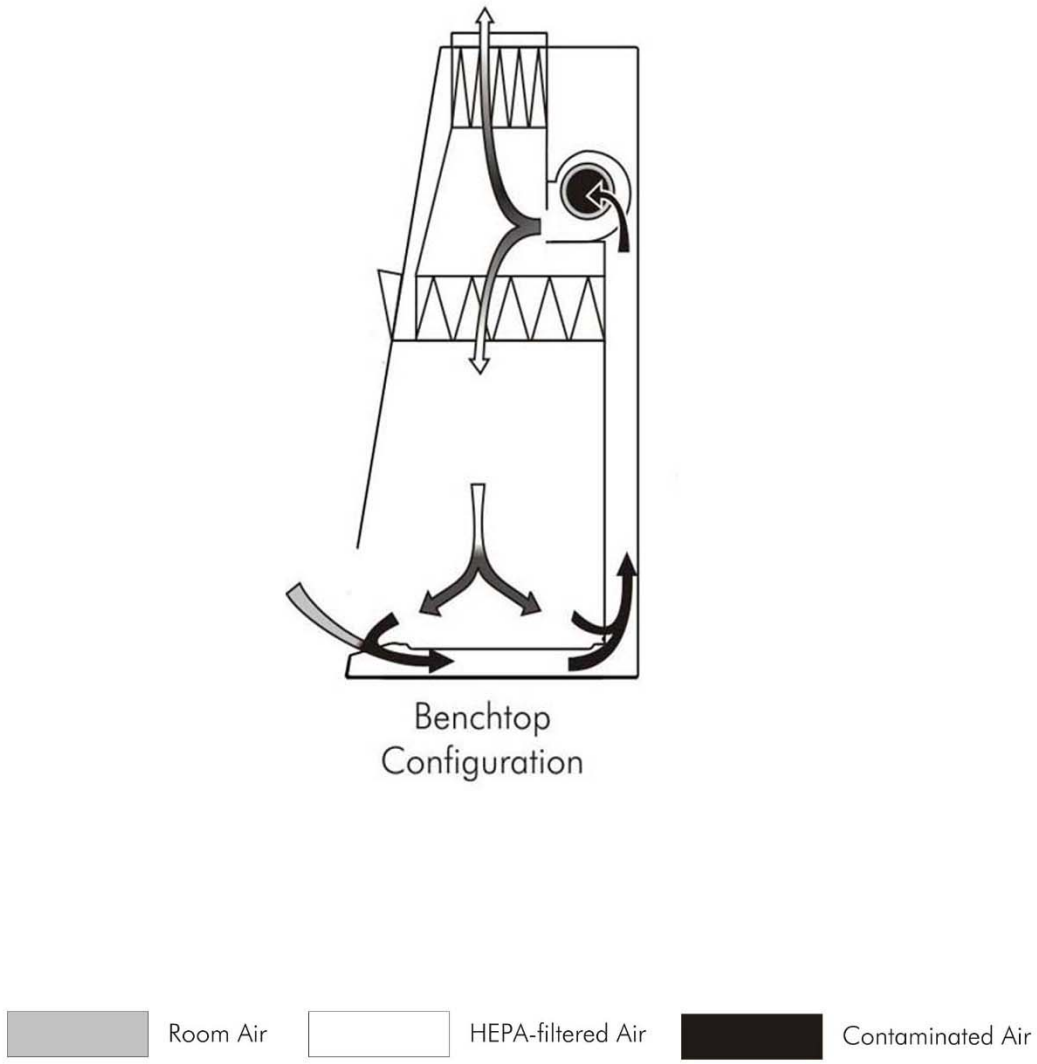
Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of antineoplastic or chemotherapeutic drugswhen connected to the mechanical exhaust system. (7,8).

Class II Type A1 biosafety cabinets:

1. Maintain minimum average inflow velocity of 75 ft/min (0.38 m/s) through the work access opening. This inflow velocity is often not adequate to provide work protection when people walk by the work opening.
2. Have HEPA/ULPA filtered downflow air that is a portion of the mixed downflow and inflow air from a common plenum (i.e., a plenum from which a portion of the air is exhausted from the cabinet with the remainder supplied to the work area).
3. May exhaust HEPA/ULPA filtered air back into the laboratory or to the environment through an external exhaust system connected to the cabinet with a canopy connection.
4. Have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.
5. May be used for work with volatile chemicals and radionuclides required as an adjunct to microbiological studies, if chemical/radionuclide risk analysis permits,when exhausted through properly functioning exhaust canopies.

Class II Type A2 biosafety cabinets:

1. Maintain a minimum average inflow velocity of 100 ft/min (0.51 m/s) through the work access opening.



2. Have HEPA/ULPA filtered downflow air that is a portion of the mixed downflow and inflow air from a common plenum.
3. May exhaust HEPA/ULPA filtered air back into the laboratory or to the environment through an external exhaust system connected to the cabinet with a canopy connection.
4. Have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.
5. May be used for work with volatile chemicals and radionuclides required as an adjunct to microbiological studies,if chemical/radionuclide risk analysis permits, when exhausted through properly functioning exhaust canopies.

Figure 7. Schematic diagram of a Class II, Type A1 and A2 biosafety cabinets
(NSF/ANSI 49-2014 graphics kindly provided by NSF International, Ann Arbor, MI, USA)

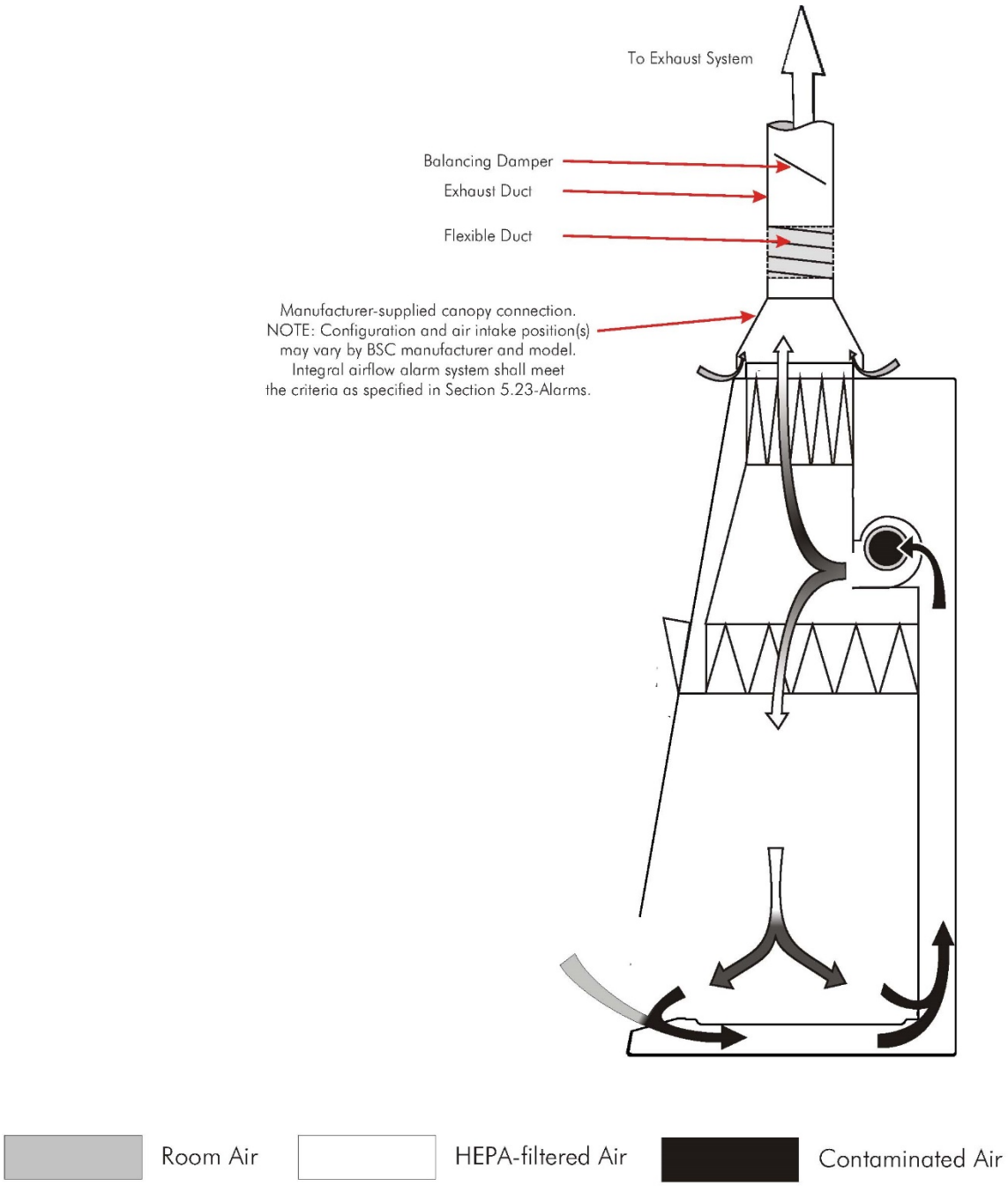


Figure 8. Schematic diagram of a Class II, Type A1 and A2 biosafety cabinet canopy exhaust

(NSF/ANSI 49-2014 graphics kindly provided by NSF International, Ann Arbor, MI, USA)

Class II Type B1 biosafety cabinets:

1. Maintain a minimum average inflow velocity of 100 ft/min (0.51 m/s) through the work access opening.
2. Have HEPA/ULPA filtered downflow air composed largely of uncontaminated recirculated inflow air.
3. Exhaust most of the contaminated downflow air to an external exhaust system through a dedicated duct connected to cabinet with a direct connection and exhausted to the atmosphere after passing through a HEPA/ULPA filter.
4. Have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.
5. May be used for work with volatile chemicals and radionuclides required as adjuncts to microbiological studiesif chemical/radionuclide risk analysis permits.

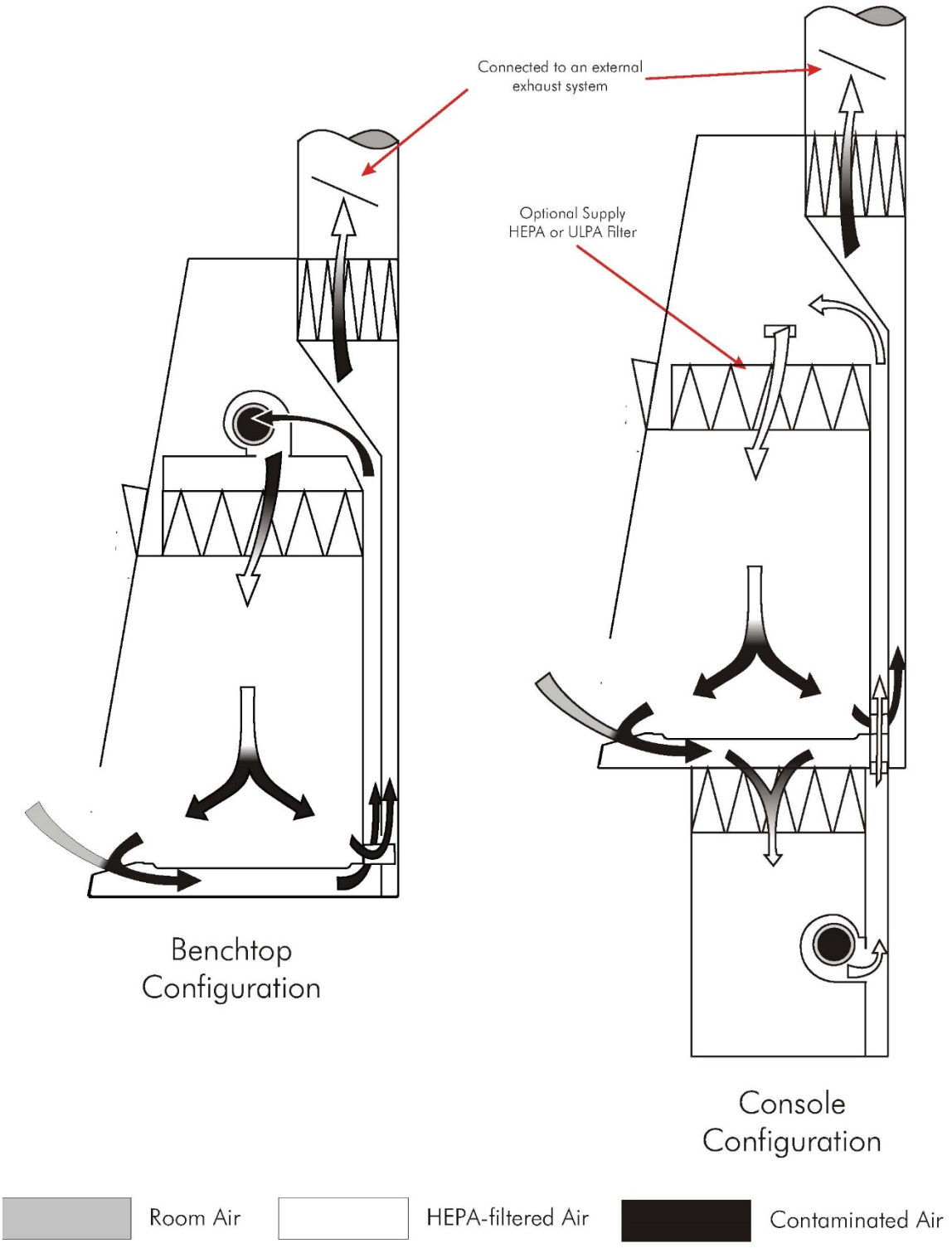


Figure 9. Schematic diagram of a Class II, Type B1 biosafety cabinet
(NSF/ANSI 49-2014 graphics kindly provided by NSF International, Ann Arbor, MI, USA)

Class II Type B2 biosafety cabinets:

1. Maintain a minimum average inflow velocity of 100 ft/min (0.51 m/s) through the work access opening.
2. Have HEPA/ULPA filtered downflow air drawn from the laboratory or the outside air (i.e., downflow air is not recirculated from the cabinet common plenum).
3. Exhaust all inflow and downflow air to the atmosphere through an external exhaust system connected to the cabinet with a direct connection after filtration through a HEPA/ULPA filter without recirculation in the cabinet or return to the laboratory.
4. Have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted (non-recirculated through the work area) negative pressure ducts and plenums.
5. May be used for work with large quantities of volatile chemicals and radionuclides required as adjuncts to microbiological studies, if chemical/radionuclide risk analysis permits.

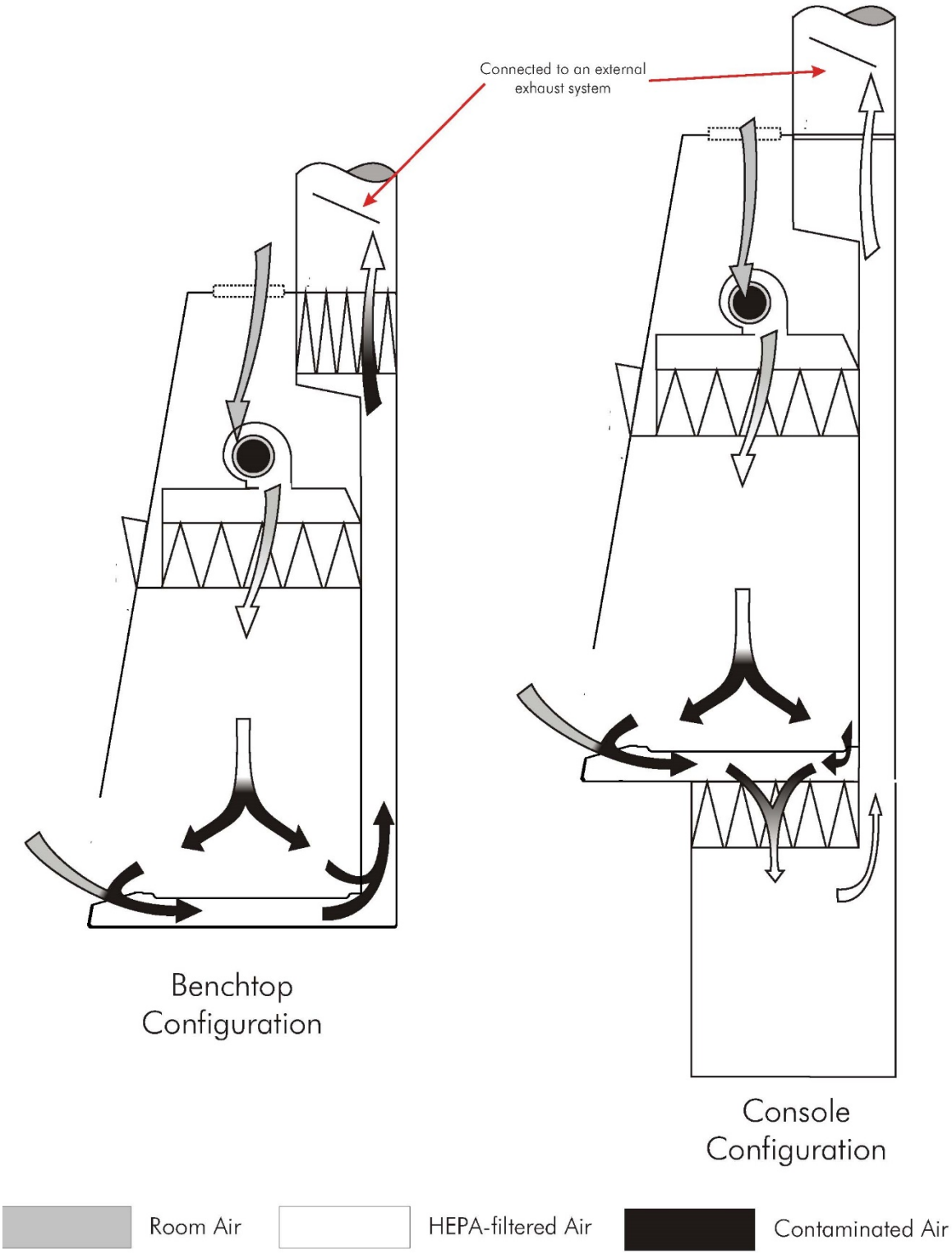


Figure 10. Schematic diagram of a Class II, Type B2 biosafety cabinet
(NSF/ANSI 49-2014 graphics kindly provided by NSF International, Ann Arbor, MI, USA)

Complete descriptions of the various Class II, Type A and Type B BSCs can be obtained fromreferences (7, 8 and 9) and from manufacturers' brochures.

Class III biosafety cabinets:

The Class III BSC was designed for work with highly infectious microbiological agents and other hazardous operations. It provides maximum protection for the environment and the worker. It is a gas-tight [no leak greater than 1x10-7 cc/s with 1% test gas at 3 inch(747 Pa) pressure water gauge] (17) enclosure with a viewing window that is secured with locks and/or requires the use of tools to open.

Access for passage of materials into the cabinet may be through any of the following: a dunk tank that is accessible through the cabinet floor; a double-door pass-through box that can be decontaminated between uses; integrated double door autoclave; or portable docking stations with double sealing connecting mechanisms that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC.

Both supply and exhaust air are HEPA/ULPA filtered. Exhaust air must pass through two HEPA/ULPA filters in series before discharge to the outdoors. Airflow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure according to manufacturer design pressure criteria. Sometimes, because of laboratory conditions, an optional exhaust fan on the BSC may be required. This exhaust fan should generally be kept separate from the exhaust fans of the facility ventilation system. The cabinet exhaust system should be equipped with an alarm system which both notifies the cabinet user and shuts down the cabinet supply and exhaust system in the event of a facility exhaust system failure.

Glove/sleeves or equivalent glove material are sealed to ports on the cabinet and allow direct manipulation of the materials isolated inside. The glove material shall be compatible with the materials being used in the cabinet. The exhaust system for the cabinet shall provide negative pressure airflow from the cabinet arm ports in case of a glove/sleeve breach. The minimum breach velocity shall be measured with a hot wire anemometer in the middle of the arm port and shall be no less than 100 ft/min (0.51 m/s). It is not a requirement that the work area be free of turbulence or cross contamination.

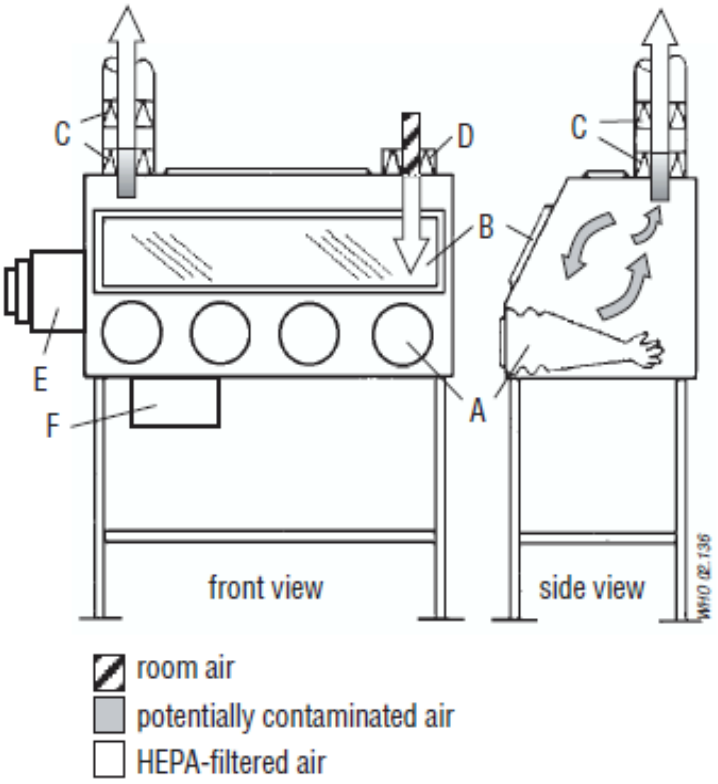


Figure 11. Schematic representation of a Class III biosafety cabinet

A, glove ports for arm-length gloves; B, sash; C, double-exhaust HEPA filters; D, supply HEPA filter; E, double-ended autoclave or pass-through box; F, chemical dunk tank. Connection of the cabinet exhaust to a dedicated, independent exhaust air system is required.

Using biosafety cabinets in the laboratory

Laboratory location of biosafety cabinets

The velocity of air flowing through the front opening into a Class II BSC is usually 100 ft/min (0.51 m/s). Currently, there are no Class II biosafety cabinets with 75 ft/min (0.38 m/s) inflow offered by BSC manufacturers. However, even with 100 ft/min (0.51 m/s) inflow velocity, theintegrity of the directional air inflow is fragile and can be easily disrupted by air currents generated by people walking close to the BSC, open windows, air supply

registersand opening and closing doors. Ideally, BSCs should be situated at a location remote from traffic and potentially disturbing air currents. A 6 in (15 cm) clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 12 in (30 cm) above the cabinet may be required for maintenance, accurate air velocity measurements across the exhaust filter and for exhaust filter changes.

All BSCs should be placed in a laboratory at a location that provides a minimum of:

1. 6 in (15 cm) from adjacent walls or columns.
2. 6 in (15 cm) between two BSCs.
3. 6 in (15 cm) space between both sides of the cabinet and 6 in (15 cm) behind the BSC to allow for service operations.
4. 40 in (102 cm) of open space in front of the BSC.
5. 60 in (152 cm) from opposing walls, bench tops and areas of occasional traffic.
6. 20 in (51 cm) between BSC and bench tops along a perpendicular wall.
7. 100 in (254 cm) between two BSCs facing each other.
8. 60 in (152 cm) from behind a doorway.
9. 40 in (102 cm) from an adjacent doorway swing side.
10. 6 in (15 cm) from an adjacent doorway hinge side.

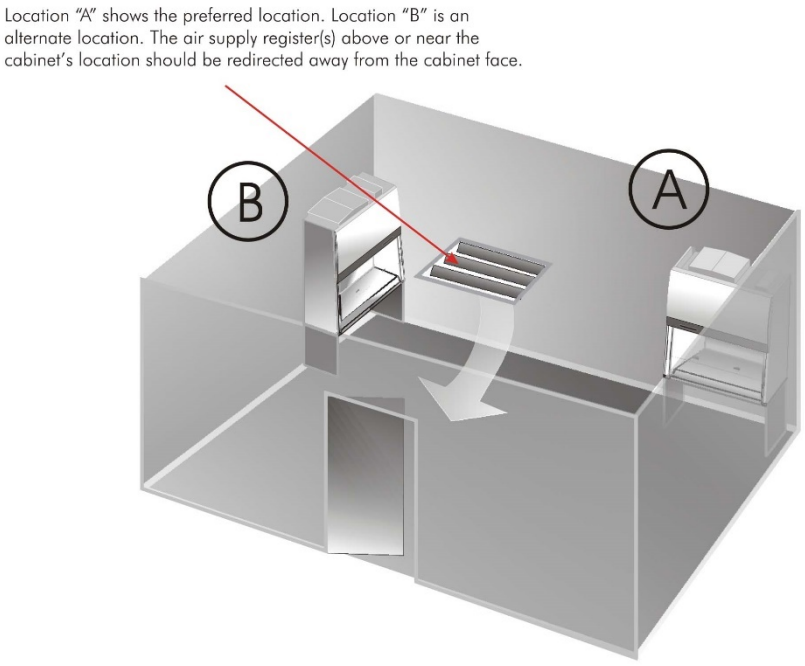


Figure 12. Suggested Laboratory Locations for Class II Biosafety Cabinets

(NSF/ANSI 49-2014 graphics kindly provided by NSF International, Ann Arbor, MI, USA)

Operations

If BSCs are not used properly, their protective benefits are greatly diminished. Operators need to be careful to maintain the integrity of the front opening air inflow when moving their arms into and out of cabinets. Arms should be moved in and out slowly, perpendicular to the front opening. Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the gloved hands and arms. The number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.

Material placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items. Materials to be placed inside the cabinet should be surface-decontaminated with an appropriate disinfectant. Work may be performed over plastic-backed paper placed on the work surface to capture splatters and splashes. All materials should be placed toward the rear of the cabinet without blocking the rear grill. All work should be performed at least 10 inches (25 cm) back from the rear of the front air intake grill. Aerosol-generating equipment (e.g. mixers, small centrifuges, etc.) should be placed toward the rear of the cabinet. Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet. Active work should flow from clean to contaminated areas across the work surface.

Discard autoclave bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to discard materials into containers outside the BSC is disruptive to the integrity of the cabinet’s air barrier and can compromise both personnel and product protection.

Type A and Type B BSC shutdown

Type A1 and A2 BSCs exhausting to the room or connected by canopy connection to laboratory mechanical exhaust should be turned off when not in use; thus extending the life of their HEPA/ULPA filters.

Type B1 and B2 BSCs are direct-ducted with dedicated exhaust ducts and dedicated roof-top exhaust fans. The roof-top exhaust fans are always on, so there is always airflow through the Type B BSCs. Some Type B BSCspermit closing the front sash while still maintaining airflow (check with the BSC manufacturer).

BSC start up procedure

1. Turn off ultraviolet (UV) lamp, if so equipped.
2. Turn on fluorescent light, inspect air intake grilles for obstructions and foreign materials and remove any obstructions.
3. Remove everything from the work surface.
4. Adjust view screen to proper height.
5. Turn on the BSC blower and allow it to run for five minutes to purge contaminants from the work area.
6. Wash hands and arms with mild, non-antimicrobial soap for 30 seconds.
7. Put on a solid front, long-sleeved gown with gathered cuffs.
8. Put on a pair of appropriate long sleeve (11 ½ - 12 inch) gloves (nitrile gloves are recommended). Consider, depending on the work procedure, disposable sleeve protectors and a second or third pair of appropriate gloves. This will minimize the shedding of skin flora into the work area and also protect hands and arms from microbial contaminationand reduce exposures by needle stick.
9. Disinfect the interior surfaces of the BSC by wiping down with appropriate disinfectant for an appropriate contact time. 70% alcohol is not considered an appropriate disinfectant because it has no effect on fungal spores.
10. Place a plastic-backed pad on the work surface without covering the air intake/exhaust grills. This will prevent spills from hitting the stainless steel surface and creating aerosols.
11. Put all items for the experiment on the BSC work surface, keeping clean items segregated from dirty items by 12 inches (30 cm). Organize the material so that dirty "contaminated" items will not be passed over (cross contaminate) clean items.
12. Exercise care that no items are placed over the front intake grill.
13. Transfer of viable materials should be performed as deeply into the cabinet (away from open face) as possible.
14. Allow air to stabilize for five minutes before starting work. This will rid the area of all "loose" contamination that may have been introduced with the items.
15. Work from "clean" to "dirty" areas and work at least ten inches (25 cm) back from rear of the front air intake grill.
16. Move arms in and out of the work access opening perpendicular to the front of the BSC in a slow steady motion to minimize disruption of the front air curtain.
17. Minimize penetration of the work opening air curtain.
18. A minimum number of needed items should be placed into the BSC to prevent overloading. Work should be planned to minimize the number of times an operator's hands and arms must enter and leave the air curtain. Ideally, have everything needed for your procedure is placed in the BSC before starting, so that nothing needs to pass in or out through the front air curtain until the procedure is completed.

19. Do not raise your hands inside the BSC above the level of the sash. If you raise your hands above the sash height, air may flow up your arms to elbows and possibly out of the BSC.
20. Know Your "Safe Working Area". A BSC safe working area is the work tray or depressed area. All work should be performed on or above the work tray. The area closer than10 inches (25 cm) from the rear of the front grill is a non-safe working area.
21. This is a general operational guideline to control airborne contaminants of low to moderate risk as stated in Technical Report No. FPS 56500000001 prepared by Dow Chemical U.S.A. for the National Cancer Institute, May 1,1972.
22. Procedure protocols defined in terms of the barrier or control concepts unique to BSC's must be developed by the laboratory workers for maximum safety and protection.
23. For preparation of antineoplastic drugs, the procedures summarized in the OSHA Technical Manual TED 1-0.15A, Section VI, Chapter 2 "Controlling Occupational Exposure to Hazardous Drugs"should be reviewed before preparing antineoplastic drugs in a BSC. https://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html

Ultraviolet lights

Germicidal ultraviolet (UV) lights are notrecommended in BSCs (NSF/ANSI 49-2014). Germicidal ultraviolet light's effectiveness is greatly reduced on dry materials including microorganisms. If UV lights are used, they must be routinely cleaned to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light germicidal radiation can be checked when the cabinet is recertified if the certifier has the equipment to measure radiation at the specific 254 ηm wavelength. Ultraviolet lights must be turned off while the room is occupied to protect eyes and skin from inadvertent exposure.

The industrial quartz on UV lamps solarizes after 6 to 9 months' operation and no longer produces 254 ηm germicidal radiation. However, the UV lamp will continue to operate without germicidal radiation for years.

Open flames

Open flames should be avoided in the environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are used. To sterilize bacteriological loops, micro burners or electric "furnaces" are available and are preferable to open flames. Disposable, sterile plastic bent rods should be used for spreading liquid culture on agar plates. Use of glass spread-plate rods decontaminated by dipping into a beaker of alcohol is not permitted.

Spills

A copy of the laboratory's protocol for handling spills should be posted, read and understood by everyone who uses the laboratory. When a spill of biohazardous material occurs within a BSC, clean-up should begin immediately while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled material should be disinfected and/or autoclaved.

Certification

The functional operation and integrity of each BSC should be certified to national performance standards at the time of installation, after internal repairs or filter replacement and regularly thereafter by qualified technicians, according to the manufacturer's instructions. Evaluation of the effectiveness of cabinet containment should include tests for cabinet integrity, HEPA/ULPA filter leaks, downflow velocity profile, face velocity, negative pressure/ventilation rate, air-flow smoke patterns, alarms and interlocks. Optional tests for electrical leaks, lighting intensity, ultraviolet light intensity, noise level and vibration may also be conducted. Special training, skills and equipment are required to perform these tests and it is highly recommended that they be performed by a qualified professional. BSCs in research laboratories are usually certified annually with a certification sticker with certification date, signature of certifier and expiration date. BSCs in clinical and hospital laboratories are certified every six months.

Surface cleaning and disinfection

All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed.

The interior surfaces of BSCs should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the work day, the final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. An appropriate disinfectant that is effective on the organisms used in the work procedures must be used. A second wiping with sterile water or 70% alcohol is needed when a corrosive disinfectant, such as bleach, is used.

The BSC blower should be running during the surface disinfection procedure. After disinfection, the blower should run for 5 minutes to purge the atmosphere inside the BSC before it is turned off.

Suggested surface disinfectants

Halogens [Hypochlorous Acid HOCl

1. Stainless steel is corroded by chlorine bleach. Sodium hypochlorite must be neutralized with sodium thiosulfate or followed by a second germicidal disinfectant.
2. 1:5 dilution of Clorox^{TM}with water (10,000 ppm) is needed to inactivate Mycobacteria in sputum.
3. 1:10 dilution with water (5,000 ppm) is commonly used. It should be made fresh monthly.
4. 1:100 dilution with water (500 ppm) must be made fresh daily and combined with a nonionic detergent. (NIH Laboratory Safety Monograph 1978)
5. 1:50 dilution stored at room temperature in a closed plastic container will deteriorate to the equivalent of a 1:100 dilution after one month (Amer. J. Infect. Control 17:1, 1989). Therefore, a 1:10 dilution remains effect for at least one month.
6. Bleach mixed with acid cleaner produces chlorine gas - 1 ppm TLV.
7. Bleach mixed with ammonia-containing cleaner produces monochloramine and dichloramine irritants.

Chlorine Dioxide

1. Tuberculocidal, Bactericidal, Virucidal and Fungicidal.

Quaternary ammonium salts

1. Each compound exhibits its own antimicrobial characteristics.
2. Chemical names of quaternary ammonium compounds used in healthcare are alkyl dimethyl benzyl ammonium chloride, alkyl didecyl dimethyl ammonium chloride, and dialkyl dimethyl ammonium chloride.
3. Newer quaternary ammonium compounds are referred to as twin-chain or dialkyl quaternaries (e.g. didecyl dimethyl ammonium bromide and dioctyl dimethyl ammonium bromide).
4. A quaternary-detergent shower is used to decontaminate BSL-4 suits when people exit maximum containment BSL-4 facilities.
5. They are the most common institutional disinfectantsand are sold under hundreds of trade names.
6. Use dilution ranges from 0.5 - 3% depending on the compound.

Phenolics

1. EPA registered as tuberculocidal.
2. Many trade names and concentrations of amylphenol and phenyl phenol.

3. Allergies, skin absorption.
4. Phenolics are not sporicidal.

Alcohols

1. Flammable.
2. Alcohols are not sporicidal and must be used as a sterile solution to prevent spread of fungal spores.
3. 70% alcohol sprayed on the work surface of an operating biosafety cabinet becomes ineffective within seconds.
4. Alcohol attacks acrylic, polypropylene, PVC and polycarbonate plastics over time.

Iodophors

1. Often used at a 0.5% concentration.
2. Wescodyne^{TM} diluted 1:200 with water is an effective BSC surface disinfectant.
3. Non-staining, nontoxic, but will leave a brown residue.
4. Active against gram negative & gram positive bacteria, viruses, fungi, yeast, M. tuberculosis and many bacterial and fungal spores.
5. Used for work surfaces, water baths and incubators.

Peroxides (stabilized) Hydrogen Peroxide 6-25%

1. Often used to sanitize surfaces in the food industry.
2. Sporicidal agent recommended by the cleanroom industry.

Peracetic Acid

1. Sporicidal agent recommended by the cleanroom industry.

BSC space decontamination

Space decontamination is mandatory when maintenance work, filter changes and performance tests require access to any contaminated interior portion of the cabinet. All work surfaces and exposed surfaces should be decontaminated with a suitable surface disinfectant before certification tests are performed and before gaseous decontamination takes place. In addition, it may be desirable to perform gaseous decontamination of the entire cabinet before performing certification tests when the cabinet has been used in a BSL-2 laboratory and is recommended when the cabinet has been used in aBSL-3 laboratory. A qualified safety and risk assessment of a BSC's potential contamination with biological agents should be performed by a biosafety officer or qualified safety professional. Appropriate decontamination (space and/or surface) should be performed

before a BSC is moved to another location. Additionally, after spills and splashes of research agents, contaminated surfaces should be suitably decontaminated.

In most instances where space decontamination is necessary, one of the procedures described below utilizing either depolymerized paraformaldehyde or chlorine dioxide gas is used. Prior to decontamination with an alternative method (such as vaporized hydrogen peroxide [VHP]), cycle parameters and validation of those parameters must be developed for each model and size of BSC. Material compatibility in terms of degradation and absorption of an alternative decontaminant are critical for maintaining cabinet integrity and the time required for decontamination, respectively. Alternate methods are required in certain instances, e.g., slow disease viruses. The decontamination method should be determined by consultation between user and certification agency. When paraformaldehyde is used for gas decontamination, follow OSHA Regulations Code of Federal Regulations, Title 29, Formaldehyde-1910-1048, which addresses monitoring; posting of regulated areas; respirator selection, protection and fit testing; medical surveillance; hazard communication, training and recordkeeping.

Personal protective equipment

Personal protective clothing should be worn when using a BSC. A solid front, back-closing laboratory gown with gathered cuffs provides better protection than front buttoning lab coat and is recommend for all BSC work. Gloves should be pulled over the cuffs of the gown rather than worn inside. Additional protection using elasticized sleeves over the gown may be worn for some procedures.Face masks and safety glasses may be required for some procedures where a splash or spray may exit the work opening.

Alarms

BSCs are equipped alarms. A sash alarm notifies the operator that the sash is not at the correct height. Airflow alarms indicate a disruption of the cabinet's normal airflow pattern. BSC training shall include procedures to be performed by operators when alarms are on.

Airflow alarms on canopy connected Type A BSCs notify the operator that the mechanical exhaust system is not functioning properly. Work may continue as long as no volatile materials are on the work surface because the BSC exhaust will be directed into the room.

Airflow alarms on Type B BSCs inform the operation that there is an immediate hazard to the operator and product because airflow within the cabinet will stop. Work should cease immediately and the laboratory supervisor should be notified. Manufacturers' instruction manuals should provide further details.

10. Safety equipment

Aerosols are important sources of infection, so precautions should be taken to reduce the extent of their formation and dispersion. Hazardous aerosols can be generated by many laboratory operations, e.g. blending, mixing, grinding, shaking, stirring, sonicating and centrifuging. Even when safe equipment is used, it is best to carry out these operations in an approved biosafety cabinet whenever possible. Biosafety cabinetuse and testing are discussed in Chapter 9.

Using safety equipment does notassure protection unless the operator is trained and uses proper techniques. Equipment should be tested regularly to ensure its continued safe performance.

Table 8 provides a checklist of safety equipment designed to eliminate or reduce certain hazards and briefly outlines their safety features. Further details on much of this equipment are given in subsequent pages. Additional information on its proper use is provided in Chapter 11.

Refer to Annex 3 for information on equipment and operations that may create hazards.

Table 8. Biosafety equipment

EQUIPMENT	HAZARD CORRECTED	SAFETY FEATURES
Biosafety cabinet		
Class I	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Does not provide product protection
Class II	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Provides product protection.
Class III	Aerosol and spatter	Maximum containment. Provides product protection.
Negative pressure flexible-film isolator	Aerosol and spatter	Maximum containment. Provides product protection.
Spatter shield	Spatter of liquids	Partial barrier between operator and work

Pipetting aids	Hazards from pipetting by mouth, e.g. ingestion of pathogens, inhalation of aerosols produced by mouth suction on pipette. Blowing liquid out of pipette or dripping from pipette. Contamination ofsuction end of pipette	Ease of use Controls contamination of suction end of pipette, protecting pipetting aid, user and vacuum line. Can be sterilized Controls leakage from pipette tip
Loop microincinerators Disposable loops		Shielded by open-ended glass or ceramic tube. Heated by gas or electricity Disposable, no heating necessary
Leakproof vessels for collection and transport of infectious materials for sterilization within a facility	Aerosols, spillage and leakage	Leakproof construction with lid or cover Durable Autoclavable
Sharps disposal containers	Puncture wounds	Puncture resistant on sides and bottom May or may not be autoclavable
Transport containersbetween laboratories and institutions	Release of microorganisms	Watertight primary and secondary containers to contain spills Absorbent material between primary and secondary container to contain spills
Autoclaves, manual or automatic	Infectious material inactivated for safe disposal or reuse	Certified design Effective moist heat sterilization
Screw-capped bottles	Aerosols and spillage	Effective containment
Vacuum line protection	Contamination of laboratory vacuum system with aerosols and overflow fluids	Cartridge-type HEPA filter prevents passage of aerosols into vacuum system. Vacuum trap flask contains appropriate disinfectant. Entire unit can be autoclaved

Negative-pressure flexible-film isolators

Negative-pressure flexible-film isolators are self-contained primary containment devices that provide protection from hazardous biological materials. Isolators may be mounted on a mobile stand. The workspace is enclosed in a transparent polyvinylchloride (PVC) envelope suspended from a steel framework. Isolators arekept at negative pressure to the surrounding environment. Supply air may pass through a HEPA filter and the workspace air is passed through a HEPA filter before exiting to the laboratory. Sometimes, HEPA-

filtered air is ducted to the building mechanical exhaust system. Isolators may be fitted with an incubator, microscope and other laboratory equipment, such as centrifuges, animal cages, heat blocks, etc. Material is introduced and removed from the isolator through sample ports without compromising microbiological integrity. Manipulations are performed using gloved sleeves sealed to the PVC. A manometer is installed to monitor envelope pressure.

Flexible-film isolators are used to manipulate high-risk organisms (Risk Groups 3 or 4) during field work where it is not feasible or appropriate to install or maintain conventional biosafety cabinets.

Pipetting aids

A pipetting aid must always be used for pipetting procedures. Mouth pipetting is strictly forbidden.

The importance of pipetting aids cannot be overemphasized. The most common hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory-associated infections.

Pathogens can also be transferred to the mouth by a contaminated finger if the finger was placed on the suction end of a pipette to hold liquid in the pipette. A lesser known hazard of mouth pipetting is the inhalation of aerosols during mouth suction of liquids. A cotton plug at the suction end of the pipettes does not effectively block passage of aerosols. If the cotton plug is tightly packed into the suction end of the pipette, strong suction may remove the plug along with aerosol and liquid. Thus, pipetting aids reduce inhalation and ingestion of pathogens.

Aerosols are also generated when a liquid is dropped from a pipette to a hard surface during procedures such as mixing cultures by alternate sucking and blowing or blowing the last drop from a pipette. Inhalation of aerosols unavoidably generated during pipetting operations can be reduced by working in a biosafety cabinet.

Pipetting aids should be selected with care. Their ergonomic design and aerosol-prevention filters are must be considered. Pipetting aids must be easy to decontaminate and clean. Plugged (aerosol-resistant) pipettes should be used when manipulating microorganisms and cell cultures.

Pipettes with cracked or chipped suction ends must not be used because they will not properly seat into the pipetting aid.

Homogenizers, shakers, blenders and sonicators

Domestic (kitchen) homogenizers are not designed to prevent release of aerosols. Use only equipment designed for laboratory procedures. Their construction minimizes or prevents such release. Bag mixers (stomachers) for large and small volumes may produce aerosols.

Homogenizers used for Risk Group 3 microorganisms should always be loaded and reopened in biosafety cabinets.

Sonicators may release large quantities of aerosol, particularly when they are improperly tuned and produce cavitation of liquids. They should be operated in biosafety cabinets or covered with shields during use. The shields and outsides of sonicators should be decontaminated after use.

Disposable transfer loops

Disposable transfer loops do not have to be sterilized. They are used once and discarded into a biohazard waste container containing disinfectant(see Chapter 2).

Micro incinerators

Electrically-heated micro incinerators have borosilicate glass or ceramic interior linings that minimize the spatter and dispersal of microbial material on the end of platinum wire loops when they are heat sterilized. Micro incineratorsused in biosafety cabinets should be placed towards the rear of the work surface to reduce disruption of airflow.

Personal protective equipment and clothing

Personal protective equipment (PPE) is the last line of defense, after administrative and engineering controls, from laboratory hazards. PPE may reduce the risk of exposure to aerosols, splashes and accidental inoculation. Risk analysis of the procedures should determine what PPE are used. Protective clothing shall always be worn when working in a laboratory and removed before leaving the laboratory. Table 9 summarizes some personal protective equipment used in laboratories and the protection afforded.

Table 9. Personal protective equipment

EQUIPMENT	HAZARD CORRECTED	SAFETY FEATURES
Laboratory coats, gowns, coveralls	Contamination of clothing	• Rear opening • Gathered cuffs • Cover street clothing
Plastic aprons	Contamination of clothing	• Waterproof
Footwear	Impact and splash	• Closed-toe
Goggles	Impact and splash	• Impact-resistant lenses (must be optically correct or worn over corrective eyeglasses) • Directly vented, indirectly vented (liquids) or non-vented (gases)
Safety glasses	Impact and spray	• Impact-resistant lenses (must be optically correct) • Side shields and brow guard (liquids)
Face shields	Impact, splash and spray	• Shield entire face from horizontal projectiles • Easily removable in case of accident
Respirators	Inhalation of aerosols	• Designs available include single-use disposable; full-face or half-face air purifying; full-face or hooded powered air purifying (PAPR); and supplied air respirators
Gloves	Hand and finger contact with microorganisms and chemicals Cuts from knives	• Disposable 11 ½ or 12 inch, long sleeve nitrile gloves are recommended for work in biosafety cabinets • Stainless steel mesh

Laboratory coats, gowns, coveralls, aprons

Laboratory coats worn in laboratories must be fully buttoned. Longsleeved, rear opening gowns or coveralls provide superior protection over laboratory coats in microbiology laboratories.

Long sleeve, rear opening gowns with gathered cuffs should be worn for work in biosafety cabinets. Aprons may be worn over laboratory coats or gowns where necessary to give

further protection against spillage of chemicals or biological materials such as blood or culture fluids.

Laundering services should be provided at/near the facility. Personnel shall not launder laboratory coats and gowns at home.

Laboratory coats, gowns, coveralls or aprons shall not be worn outside laboratory areas.

Goggles, safety glasses, face shields, face masks

The choice of eye and face protective equipment to protect the eyes and face from splashes and impacting objects will depend on the activities performed.

Prescription or non-prescription safety glasses haveside shields, brow guards and special frames to accept front-mounted shatterproof lenses to prevent eye injury when objects impact the lenses.

Safety glasses usually have side guards and may have brow guards to reduce migration of liquids sprayed on the brow into the eyes.

Goggles should be worn for splash and impact protection. Safety glasses do not provide adequate splash protection. Goggles can be worn over eye glasses and contact lenses (which do not provide protection against biological or chemical hazards). There are three types of goggles; direct vented (solid objects), indirect vented (liquids) and non-vented (gases).

Face shields (visors) are made of shatterproof plastic, fit over the face and are held in place by head straps or caps. Face shields are designed to protect the wearer from objects coming horizontally toward the face. For example, an exploding cryovial that has just been removed from liquid nitrogen storage.

Face masks and surgical masks in combination with safety glasses may be used to reduce face and eye exposure to liquid droplets. Plastic visors are attached to some face masks to provide additional eye protection. They do not provide respiratory protection.Face masks are also recommended for individuals working with animals to reduce occupational allergies.

Goggles, safety glasses, face shields and reusable respirators must be decontaminated after use and shall not be worn outside laboratory areas.

Respirators

Respiratory protection is needed when working with biological aerosols and volatile chemicals when engineering controls are not available (e.g. cleaning up a spill of infectious material or working with potential aerosol-generating procedures outside of primary containment).

The choice of respirator will depend on the type of hazard(s). Respirators are available with interchangeable filters for protection against gases, vapors, particulates and microorganisms. It is imperative personnel have respirators that are properly fit tested to their face and that the respirator filter media is appropriate to the type of work being done.

Self-contained full-face respirators with an integral air supply provide full protection. Positive air pressure powered respirators (PAPRs) with HEPA filters also provide full protection from aerosols and are becoming common for certain research and clinical applications. Advice from a suitably qualified person (e.g. an occupational or industrial hygienist) is required to ensure selection of an appropriate respirator. Single-use disposable respirators (ISO 13.340.30), such as N-95 respirators,have been designed to provide some protection from exposures to small particles and some biological materials.

Respirators shall not be worn outside the laboratory areas.

Gloves

Hands become contaminatedduring mostlaboratory procedures. Hands are also vulnerable to “sharps” injuries. Disposable nitrile gloves are recommended for general laboratory work, handling infectious materials and blood and body fluids. Nitrile gloves are more resistant than latex gloves for the types of chemicals used in biomedical research. Disposable 11 ½ or 12 inch, long sleeve nitrile gloves are recommended for work in biosafety cabinets.

Reusable utility gloves are used for washing and handling large, potentially contaminated equipment. Utility gloves must be decontaminated and cleaned before reuse.

Gloves shall be removed and hands thoroughly washed after handling infectious materials, working in a biosafety cabinet, after each procedure and before leaving the laboratory. Used disposable gloves shall be discarded as biohazard or medical waste.

Stainless steel mesh or Kevlar padded gloves may be worn when there is a potential exposure to sharp instruments, e.g. during postmortem examinations. Such gloves protect against slicing motion but do not protect against puncture injury.

No gloves protect from needle sticks – Two pairs of gloves provide additional protection from needle sticks.

Gloves should not be worn outside the laboratory areas.

For further information, see references (12, 17 and 18).

PART IV

Standard microbiological practices

11. Laboratory techniques

Human error, poor laboratory technique and improper use of equipment causes the majority of occupationally acquired laboratory exposures and injuries. This chapter provides a compendium of technical methods that may help workers avoid or minimize the most commonly reported occupational exposures and injuries.

Safe handling of specimens in the laboratory

Improper collection, transport and handling of specimens in the laboratory carries a risk of infection to the personnel involved.

Specimen containers

Primary specimen containers may be glass or preferably plastic. They should be robust and should not leak when the cap or stopper is correctly applied. No material should remain on the outside of the container. Containers should be correctly labeled to facilitate identification. Specimen request or specification forms should not be wrapped around the primary containers but placed in separate, preferably waterproof envelopes.

Transport of specimens within the facility

To avoid accidental leakage or spillage, sealable secondary containers made of metal, reusable plastic or sealable single-use plastic bags must be used to transport primary specimen containers.Secondary containers may be fitted with racks to keep specimen containers upright. Metal or reusable plastic secondary containers should be autoclavable and resistant to chemical disinfectants. Reusable secondary containers should be regularly decontaminated.

Receipt of specimens

Laboratories that receive large numbers of specimens should designate a particular room or area for this purpose.

Opening packages

Personnel who receive and unpack specimens should be aware of the potential health hazards involvedand have standard precautions (21) training. Broken or leaking containers

present a potential hazard. Primary specimen containers should be opened in a biosafety cabinet and appropriate disinfectants should be available

Use of pipettes and pipetting aids

1. Pipetting aids must always be used. Pipetting by mouth is prohibited.
2. All pipettes must have cotton plugs to reduce contamination of pipetting devices.
3. Air should never be blown through liquids containing potentially infectious materials.
4. Infectious materials should not be mixed by alternate suction and expulsion througha pipette.
5. Liquids must not be forcibly expelled from pipettes.
6. Mark-to-mark pipettes are preferable to blowout pipettes.
7. Contaminated pipettes must be completely submerged in a suitable disinfectantcontained in an unbreakable, horizontal container. Pipettes must remain in the disinfectant foran appropriate length of time before disposal.
8. A pipette discard container must be placed on the work surface of a biosafety cabinet,not outside the BSC.
9. Syringes fitted with hypodermic needles must not be used for pipetting.
10. Devices for opening septum-capped bottles should be used if pipettes are used to remove liquids. Use of hypodermic needles and syringes should be avoided if possible. A gauze pad should be held over the septum when using a needle to remove liquid.
11. To avoid dispersion of infectious material dropped from a pipette, plastic backed paper should be placed on the BSC work surface and be discarded as infectious waste after use.

Avoiding the dispersal of infectious materials

1. To avoid premature dropping of viable microorganisms, microbiological transferloops should have a diameter of 2–3 mm and be completely closed. The shanksshould be not more than 6 cm long to minimize vibration.
2. The risk of spatter of infectious material by open Bunsen burner flames shouldbe avoided by using an enclosed electric microincinerator to sterilize transfer loops.Disposable transfer loops, which do not need to be re-sterilized, are preferable.
3. Care should be taken when drying sputum samples to avoid creating aerosols.
4. Discarded specimens and cultures for autoclaving and/or disposal should be placedin leak-proof containers, e.g. laboratory biohazard bags or stainless steel buckets. Specimen tops should be secured (e.g.with autoclave tape) prior to disposal into waste containers.

5. Work areas must be decontaminated with a suitable disinfectant at the end ofeach work period.

For further information, see reference (12).

Use of biosafety cabinets (BSCs)

The use and limitations of biosafety cabinets should be explained to allpotential users (see Chapter 9), with reference to national standards and relevantliterature. Written protocols or safety or operations manuals should be issued tostaff. In particular, it must be made clear that the biosafety cabinet will not protect theoperator from spillage, breakage or poor technique.

1. The cabinet must not be used if it is not working properly.
2. The glass viewing panel (sash) must remain at the proper height and not be moved when the cabinet is in use.
3. Apparatus and materials in the cabinet must be kept to a minimum. Air circulation at the front and rear intake grills must not be blocked.
4. Bunsen burners must not be used in biosafety cabinets. The heat produced will distort the airflow and may damage the filters. An electric microincinerator is permissible but sterile disposable transfer loops are preferable.
5. All work must be carried out in the middle or rear part of the work surface.
6. Operators must view the work surface through the glass sash.
7. Traffic behind the operator should be minimized.
8. The operator should not disturb the airflow by repeated removal and reintroduction of his or her arms.
9. Air intake grills must not be blocked with notes, pipettes or other materials because airflow will be disrupted and may cause potential contamination of work material and operator exposure.
10. Paperwork should never be placed inside biosafety cabinets.
11. The interior of biosafety cabinets should be wiped using an appropriate disinfectant after work is completed and at the end of the day.
12. The cabinet blower fan should run for at least 5 minutesafter materials are placed in the cabinet before beginning work.
13. The cabinet blower fan should run for at least 5 minutes after completion of work, removal of supplies and equipment and disinfection of the interior surfaces.

For further information about biosafety cabinets see Chapter 9.

Avoiding ingestion of infectious materials and contact with skin and eyes

1. Large particles and droplets (> 5 µm in diameter) released during microbiological manipulations settle rapidly on bench surfaces and on the hands of the operator.
2. Long sleeve nitrile disposable gloves and gowns with gathered cuffs should be worn.
3. Laboratory workers must avoid touching their mouth, eyes and face. This is a very common source of laboratory-acquired infection.
4. Food and drink must not be consumed or stored in the laboratory.
5. No articles shall be placed in the mouth. No pens, pencils or chewing gum.
6. Cosmetics shall not be applied in the laboratory.
7. The face, eyes and mouth should be shielded or otherwise protected during any operation that may produce splashes of potentially infectious materials.

Avoiding injection of infectious materials

1. Accidental inoculation resulting from injury with broken or chipped glassware can be avoided by using careful practices and procedures. Glassware should be replaced with plasticware whenever possible.
2. Accidental injection may result from sharps, e.g. hypodermic needles (needlesticks), glass Pasteur pipettes, glass microscope slides, glass coverslips, broken glass and micropipette tips.
3. Needlesticks can be reduced by: (a) minimizing the use of syringes and needles (e.g. special devices for opening flame-sealed ampules and septum bottles) so that pipettes can be used instead of syringes and needles; or (b) using engineered sharp safety devices when syringes and needles are necessary.
4. Needles should never be recapped.
5. Disposable sharps should be discarded into puncture-resistant sharps containers that can be closed when three-quarters full.
6. Glass Pasteur pipettes should be replacedwith plastic Pasteur pipettes.

Separation of serum

1. Only properly trained staff should perform this work.
2. Gloves and eye and mucous membrane personal protective equipment shall be worn.
3. Splashes and aerosols can be reduced by good laboratory technique. Blood and serum should be pipetted carefully, not poured. Pipetting by mouth is forbidden.

4. After use, pipettes must be completely immersed in an appropriate disinfectant. They should remain in the disinfectant for an appropriate time before disposal or washing and sterilization for reuse.
5. Discarded specimen tubes containing blood clots, etc. (with caps replaced) should be placed in suitable leak proof containers for autoclaving and/or incineration.
6. Suitable disinfectants should be available for clean-up of splashes and spillages (see Chapter 13).

Use of centrifuges

1. Satisfactory mechanical performance of laboratory centrifuges is a requirement for microbiological safety.
2. Centrifuges should be calibrated and operated according to the manufacturer's instructions.
3. Ultracentrifuges must have a log book near the centrifuge to record run times and speeds for each rotor serial number.
4. Centrifuges should be at a level that allows workers to see into the centrifuge chamber for placement of tubes, centrifuge heads or buckets.
5. Centrifuge tubes and specimen containers should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use.
6. Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation.
7. Centrifuge heads and buckets must be loaded and opened in a biosafety cabinet whenever possible.
8. Buckets and trunnions should be paired by equal weight and balanced with tubes in place.
9. The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should follow the manufacturer's instructions.
10. Laboratory grade water should be used to balance empty buckets. Saline or hypochlorite solutions corrode metals and should not be used.
11. Sealable centrifuge buckets (safety cups) must be used for potentially infectious microorganisms.
12. When using angle-head centrifuge rotors, care must be taken to ensure that tubes are not overloaded to prevent leaks.
13. The interior of the centrifuge chamber should be inspected daily for stains, spills or soiling. If staining or soiling ispresent, the chamber should be disinfected and centrifugation protocols should be re-evaluated.
14. Centrifuge rotors and buckets should be inspected daily for signs of corrosion and for hair-line cracks.
15. Buckets, rotors and centrifuge chambers should be decontaminated after each use, rinsed and stored in an inverted position to drain liquid.
16. Infectious aerosols may be produced during centrifugation if there are leaks of fluid from centrifuge heads or buckets. Enclosing centrifuges in Class III biosafety

cabinets will prevent dispersion of aerosols. However, good centrifuge technique, rotors with safety gaskets and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.

Use of homogenizers, shakers, blenders and sonicators

1. Domestic (kitchen) homogenizers should not be used in laboratories because they mayleak and release aerosols. Laboratory blenders and bag mixers (stomachers) are safer.
2. Caps and cups or bottles should be in good condition and free from flaws ordistortion. Caps should be well-fitting and gaskets should be in good condition.
3. Pressure builds up in the chamber during the operation of homogenizers, shakersand sonicators. Aerosols containing infectious materials may escape from the cap orchamber. Plastic, particularly polytetrafluoroethylene (PTFE),chambersare recommended because glass may break and release infectious material andpossibly injure the operator.
4. When in use, homogenizers, shakers and sonicators should be covered by a strongtransparent plastic casing. This should be disinfected after use. Where possible, thesemachines should be operated under their plastic covers in a biosafety cabinet.
5. At the end of the operation the chamber should be opened in a biosafetycabinet.
6. Hearing protection should be provided for people using sonicators.

Use of tissue grinders

1. Glass grinders should be held in absorbent material in a gloved hand. Plastic polytetrafluoroethylene (PTFE)grinders are safer.
2. Tissue grinders should be operated and opened in a biosafety cabinet.

Care and use of refrigerators and freezers

1. Refrigerators, deep-freezers and solid carbon dioxide (dry-ice) chests should bedefrosted and cleaned periodically and broken ampoules, tubes, etc. removed.
2. Face protection and utility plastic gloves should beworn during cleaning. After cleaning, the inner surfaces of the cabinet should bedisinfected.
3. All containers stored in refrigerators, etc. should be clearly labeled with the scientific name of the contents, the date stored and the name of the individual who stored them. Unlabeled and obsolete materials should be autoclaved and discarded.
4. An inventory must be maintained of the freezer’s contents.
5. Large quantities of flammable solutions must not be stored in a refrigerator unless it is explosionproof. Notices to this effect should be placed on refrigerator doors.

Opening ampoules containing lyophilized infectious materials

Care should be taken when glass ampoules of freeze-dried materials are opened because the contents may be under reduced pressure and a sudden inrush of air may disperse some of the materials into the laboratory. Ampoules should always be opened in a biosafety cabinet. The following procedures are recommended for opening ampoules:

1. First decontaminate the outer surface of the ampoule.
2. The recommended procedure is to use a device specifically designed for opening ampoules.
3. Make a file mark on the tube near to the middle of the cotton or cellulose plug, if present.
4. Hold the ampoule in disinfectant-soaked gauze to protect hands before breaking it at a file scratch.
5. Remove the top gently and treat as contaminated material.
6. If the plug is still above the contents of the ampoule, remove it with sterile forceps.
7. Add liquid for resuspension slowly to the ampoule to avoid frothing.

Storage of ampoules containing infectious materials

Glass ampoules containing infectious materials should never be immersed in liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode when removed. If very low temperatures are required, glass ampoules should be stored only in the gaseous phase above the liquid nitrogen. Alternatively, plastic, double-gasketed cryovials containing infectious material can be stored in liquid nitrogen. Otherwise, infectious materials should be stored in ultra-low freezers or on dry ice. Laboratory workers should wear eye and hand protection when removing ampoules from cold storage.

The outer surfaces of glass ampoules or cryovials should be disinfected when they are removed from storage.

Standard precautions with blood and other body fluids, tissues and excreta

Standard precautions (which include “universal precautions” (21) are designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection.

Collection, labeling and transport of specimens

1. Standard precautions (21) should always be followed; gloves should be worn for all procedures.
2. Blood should be collected from patients and animals by trained staff.
3. For phlebotomies, conventional needle and syringe systems should be replaced by single-use safety vacuum devices that allow the collection of blood directly into stoppered transport and/or culture tubes, automatically disabling the needle after use.
4. The tubes should be placed in secondary containers for transport to the laboratory (see Chapter 14 for transport requirements) and within the laboratory facility (see section on Transport of specimens within the facility in this chapter). Request forms should be placed in separate waterproof bags or envelopes.
5. Reception staff should not open these bags.

Opening specimen tubes and sampling contents

1. Specimen tubes should be opened in a biosafety cabinet.
2. Gloves and laboratory gown or lab coat must be worn.
3. Eye and mucous membrane protection is required when opening specimens on the bench top. A plastic splash shield on the bench may reduce exposures when tubes are opened behind the shield.
4. Protective clothing should be supplemented with a plastic apron.
5. The stopper should be grasped through a piece of disinfectant-soaked gauze to prevent splashing.

Glass and “sharps”

1. Plastics should replace glass wherever possible. Only laboratory grade (borosilicate) glass should be used and any article that is chipped or cracked should be discarded.
2. Hypodermic needles must not be used as pipettes (see also section on Avoiding injection of infectious materials in this chapter).

Films and smears for microscopy

Fixing and staining blood, sputum and fecal samples for microscopy may notnecessarily kill all organisms or viruses on the smears. These items should be handledwith gloves, stored appropriately and decontaminated and/or autoclaved beforedisposal.

Automated equipment (sonicators, vortex mixers)

1. Equipment should be closeable to avoid dispersion of droplets and aerosols.
2. Effluents should be collected in closed vessels for disinfection or autoclaving before disposal.
3. Equipment should be disinfected at the end of each task following manufacturers'instructions.

Tissue sectioning

1. Formalin fixed tissue should be used whenever possible.
2. Frozen sectioning should be avoided. When necessary, the cryostat should beshielded and the operator should wear a face shield or other eye, face and mucus membrane protection.
3. For decontamination,the temperature of the instrument should be raised to at least 20°C or higher. Some cryotomes have a built in heat decontamination cycle.

Decontamination

1. Sodium hypochlorite (bleach) diluted 1:10 with water or an appropriate disinfectant, such a quaternary ammonium or an iodophore, is recommended for surface decontamination.
2. High-level sterilants, such as glutaraldehyde, are recommended for decontamination of certain reusable medical devices such as endoscopes. Sterilants must be used in closed containers, not on surfaces(see Chapter 13).

Precautions with materials that may contain prions

Prions (also referred to as "slow viruses") are associated with the transmissible spongiform encephalopathies (TSEs), notably Creutzfeldt-Jakob disease (CJD; including the new variant form), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and Kuru in humans; scrapie in sheep and goats; bovine spongiform encephalopathy (BSE) in cattle; and other transmissible encephalopathies of deer, elk and mink.

Although CJD has been transmitted to humans, there appear to be no proven cases of laboratory-associated infections with any of these agents. Nevertheless, it is prudent to observe certain precautions when handling of material from infected or potentially infected humans and animals.

The selection of a biosafety level for work with materials associated with TSEs will depend on the nature of the agent and the samples to be studied and should be undertaken in consultation with national authorities. The highest concentrations of prions are found in

central nervous system tissue. Animal studies suggest that it is likely that high concentrations of prions may also be found in the spleen, thymus, lymph nodes and lung. Recent studies indicate that prions in lingual and skeletal muscle tissue may also present a potential infection risk (20–23).

Because complete inactivation of prions is difficult to achieve, it is important to stress the use of disposable instruments whenever possibleand to use a disposable plastic-backed paper covering on the work surface of a biosafety cabinet.

The main precaution is to avoid ingestion, mucous membrane exposure or puncture of the laboratory worker's skin. The following additional precautions should be taken because prions are not inactivated by the usual laboratory disinfection and sterilization procedures.

1. The use of dedicated equipment, i.e. equipment not shared with other laboratories, is highly recommended.
2. Disposable laboratory protective clothing (long sleeve gowns and aprons) and long sleeve gloves must be worn (steel mesh gloves between rubber gloves for pathologists).
3. Use of disposable plasticware, which can be treated and discarded as dry infectious waste, is highly recommended.
4. Tissue processors should not be used because of the problems of disinfection.
5. Plastic jars and beakers should be used instead of glass.
6. All manipulations must be conducted in biosafety cabinets.
7. Great care should be exercised to avoid aerosol production, ingestion and cuts and punctures of the skin.
8. Formalin-fixed tissues shall be regarded as infectious, even after prolonged exposure to formalin.
9. Histological thin sections containing prions can be inactivated after exposure to 96% formic acid for 1 h (24, 25).
10. Bench waste, including disposable gloves, gowns and aprons, should be autoclaved using a pre-vacuum autoclave at 134–137 °C for a single cycle of 1-hour minimum, followed by incineration.
11. Non-disposable instruments, including steel mesh gloves, must be collected for decontamination.
12. Infectious liquid waste contaminated with prions should be treated with 1N NaOH or bleach diluted 1:2, final concentration, for 1 hour.
13. Paraformaldehyde vaporization procedures do not inactivate prions. Prions are not affected by germicidal ultraviolet irradiation.
14. Biosafety cabinets must still be space decontaminated by standard methods (i.e. formaldehyde gas, chlorine dioxide gas or vaporized hydrogen peroxide) to inactivate other microorganisms that may be present on HEPA/ULPA filters.

15. Prion-contaminated biosafety cabinet work surfaces and other surfaces can be decontaminated with 1N NaOH or sodium hypochlorite (bleach) diluted 1:2 containing available chlorine at 20 g/l (2%).
16. High-efficiency particulate air (HEPA) filters should be incinerated at a minimum temperature of 1000 °C after removal. Recommended additional steps prior to incineration include:
    a. spraying of the exposed face of the filter with lacquer hairspray prior to removal,
    b. filters should be “bagged out” of filter holders, or
    c. removal of the HEPA filters from the working chamber so that the inaccessible plenum of the cabinet is not contaminated.
17. Steel instruments should be placed in 1N sodium hydroxide and autoclaved for 1 hour. Stainless steel buckets containing the NaOH and instruments must cool for at least an hour before being handled.
18. Instruments that cannot be autoclaved can be cleaned by repeated wetting with 1N sodium hydroxide or bleach diluted 1:2 over a 1-h period. Appropriate washing to remove residual sodium hydroxideor bleach is required.

For further information on the handling of unconventional agents see references (12, 26 and 27).

12. Contingency plans and emergency procedures

Every laboratory that works with infectious microorganisms should institute safety precautions appropriate to the hazard of the organisms and the animals being handled.

All significant problems, violations of the Biosafety Guidelines or any significant research-related accidents and illnesses should be reported to the Biomedical Research Department, Ministry of Public Health (MOPH) and related national emergency services. A full report should be prepared by the biosafety officer and submitted to the MOPH with the names of the persons involved and the contingency plan followed.

A written contingency plan for dealing with laboratory and animal facility accidents is required for any facility that works with or stores Risk Group 3 or 4 microorganisms (high containment laboratory – Biosafety Level 3 and maximum containment laboratory – Biosafety Level 4). National and/or local health authorities should be involved in the development of the emergency preparedness plan.

Contingency plan

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake and explosion.
2. Biohazard risk assessments.
3. Incident-exposure management and decontamination.
4. Emergency evacuation of people and animals from the premises.
5. Emergency medical treatment of exposed and injured persons.
6. Medical surveillance of exposed persons.
7. Clinical management of exposed persons.
8. Epidemiological investigation.
9. Post-incident continuity of operationsplans.

During development of this plan, the following items should be considered for inclusion:

1. Identification of high-risk organisms.
2. Location of high-risk areas, e.g. laboratories, storage areas and animal facilities.
3. Identification of at-risk personnel and populations.

4. Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists and fire and police services.
5. Lists of treatment and isolation facilities that can receive exposed or infected persons.
6. Transport of exposed or infected persons.
7. Lists of sources of immune serum, vaccines, drugs, special equipment and supplies.
8. Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

Emergency procedures for microbiological laboratories

Puncture wounds, cuts and abrasions

The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant and seek medical attention as necessary. The cause of the wound and the organisms involved should be reported and appropriate and complete medical records kept.

Ingestion of potentially infectious material

Protective clothing should be removed and medical attention sought. Identification of the material ingested and circumstances of the incident should be reported and appropriate and complete medical records kept.

Potentially infectious aerosol release (outside a biosafety cabinet)

All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. The laboratory supervisor and the biosafety officer should be informed immediately. Nobody should enter the room for an appropriate amount of time (e.g. 1 hour) to allow aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed (e.g. for 24 hours).

Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the biosafety officer. Appropriate protective clothing and respiratory protection should be worn.

Broken containers and spilled infectious substances

Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels. Disinfectant should then be poured over these and left for the appropriate amount of time. The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be flooded with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant. Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container. Gloves should be worn for all of these procedures.

If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminatedwaste container.

Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets

If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow droplet settling. If a breakage is discovered after the machine has stopped, the lid should be immediatelyclosed and left closed (e.g. for 30 min). In both instances, the biosafety officer should be informed.

Strong (e.g. thick plastic or rubber) gloves, covered if necessary with suitable disposable gloves, should be worn for all subsequent operations. Forceps or cotton held in forceps should be used to retrieve glass debris.

All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant known to be active against the organisms concerned (see Chapter 13). Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.

The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

Breakage of tubes inside sealable buckets (safety cups)

All sealed centrifuge buckets should be loaded and unloaded in a biosafety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.

Fire and natural disasters

Fire and other services should be involved with development of emergency preparedness plans. They should be told in advance which rooms contain potentially infectious materials. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.

After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Infectious materials should be collected in leak proof boxes or strong disposable bags.

Salvage or final disposal should be determined by biosafety staff on the basis of local ordinances.

Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed in the facility:

1. The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called).
2. Director of the institution or laboratory.
3. Laboratory supervisor.
4. Biosafety office.
5. Biomedical Research Department in Ministry of Public Health.
6. Fire services.
7. Hospitals/ambulance services/medical staff (names of individual clinics, departments, and/or medical staff, if possible).
8. Police.
9. Medical officer.
10. Responsible technician.
11. Water, gas and electricity services.

Emergency equipment

The following emergency equipment must be available:

1. First aid kit, including universal and special antidotes.
2. Appropriate fire extinguishers and fire blankets.

The following are also suggested but may be varied according to local circumstances:

1. Full protective clothing (one-piece coveralls, gloves and head covering – for incidents involving microorganisms in Risk Groups 3 and 4).
2. Full-face respirators or PAPRs with appropriate chemical and particulate filter canisters.
3. Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers.
4. Stretcher.
5. Tools, e.g. hammers, axes, spanners, screwdrivers, ladders and ropes.
6. Hazard area demarcation equipment and notices.

For further information, see references (12and 28).

13. Disinfection and sterilization

A basic knowledge of disinfection and sterilization is crucial for biosafety in the laboratory. Since heavily soiled items cannot promptly be disinfected or sterilized, it is equally important to understand the fundamentals of cleaning prior to disinfection (pre-cleaning). In this regard, the following general principles apply to all known classes of microbial pathogens.

Specific decontamination requirements will depend on the type of experimental work and the nature of the infectious agent(s) being handled. The generic information given here can be used to develop both standardized and more specific procedures to deal with biohazard(s) involved in a particular laboratory.

Contact times for disinfectants are specific for each material and manufacturer. Therefore, all recommendations for use of disinfectants should follow manufacturers' specifications.

Definitions

Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

Antimicrobial – An agent that kills microorganisms or suppresses their growth and multiplication.

Antiseptic– A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

Biocide- A general term for any agent that kills organisms.

Chemical germicide – A chemical or a mixture of chemicals used to kill microorganisms.

Decontamination – Any process for reducing, removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

Disinfectant– A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

Disinfection – A physical or chemical means of killing microorganisms, but not necessarily spores.

Microbicide– A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial”.

Sporicide – A chemical or mixture of chemicals used to kill microorganisms and spores.

Sterilization – A process that kills and/or inactivates all classes of microorganisms and sporeswith a probability of 1 in a million that one organism will survive the process.

Cleaning laboratory materials

Cleaning is the removal of dirt, organic matter and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent. Dirt, soil and organic matter can shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides and disinfectants).

Pre-cleaning is essential to achieve proper disinfection or sterilization. Many germicidal products claim activity only on pre-cleaned items. Pre-cleaning must be carried out with care to avoid exposure to infectious agents.

Materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for pre-cleaning and disinfection.

Chemical germicides

Many types of chemicals can be used as disinfectants and/or antiseptics. Since there is an ever-increasing number and variety of commercial products, formulations must be carefully selected for specific needs.

The germicidal activity of many chemicals is faster and better at higher temperatures. At the same time, higher temperatures can accelerate their evaporation and also degrade them. Particular care is needed for the use and storage of such chemicals in tropical regions where their shelflife may be reduced because of high ambient temperatures.

Many germicides can be harmful to humans or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers’ instructions. For personal safety, gloves, gowns or aprons and eye protection are recommended when preparing dilutions of chemical germicides.

Chemical germicides are generally not required for routine cleaning of floors, walls, equipment and furniture. However, their use may be appropriate for certain cases of outbreak control.

Proper use of chemical germicides will contribute to workplace safety while reducing the risk from infectious agents. As far as possible, the number of germicidal chemicals to be used should be limited for economic reasons, inventory control and to limit environmental pollution.

Commonly used classes of chemical germicides are described below, with generic information on their applications and safety profiles. Unless otherwise indicated, the germicide concentrations are given in weight/volume (w/v). Table 10 summarizes the recommended dilutions of chlorine-releasing compounds.

Table 10. Recommended dilutions of chlorine-releasing compounds

	"CLEAN" CONDITIONS (a)	"DIRTY" CONDITIONS (b)
Available chlorine required	0.1% (1 g/l)	0.5% (5 g/l)
Sodium hypochlorite solution (5% available chlorine)	20 ml/l	100 ml/l
Calcium hypochlorite (70% available chlorine)	1.4 g/l	7.0 g/l
Sodium dichloroisocyanurate powder (60% available chlorine)	1.7 g/l	8.5 g/l
Sodium dichloroisocyanurate tablets (1.5 g available chlorine per tablet)	1 tablet per liter	4 tablets per liter
Chloramine (25% available chlorine) (c)	20 g/l	20 g/l

a After removal of bulk material.
b For flooding, e.g. on blood or before removal of bulk material.
c See text

Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available broad-spectrum chemical germicide. It is normally sold as household bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine.

Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g. with or without a lid) and size of their containers, the frequency and nature of use and ambient conditions. As a

general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week or longer, depending on their concentration.

A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine and a detergent. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solution (household bleach) contains 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively. Diluted chlorine solutions should be stored in closed bottles and not exposed to heat or sunlight. Industrial solutions of bleach have a sodiumhypochlorite concentration of nearly 120 g/l and must be diluted accordingly to obtain the levels indicated above.

Granules or tablets of calcium hypochlorite ($Ca(ClO)_2$) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l, will then contain 1.0 g/l and 5 g/l available chlorine, respectively.

Bleach is not recommended as an antiseptic, but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. In emergencies, bleach can also be used to disinfect water for drinking, with a final concentration of 1–2 mg/l available chlorine. Chlorine-containing solutions must never be autoclaved.

Chlorine gas is highly toxic. Bleach must therefore be stored and used in wellventilated areas only. Also, bleach must not be mixed with acids that cause rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, in particular bleach, should be avoided.

Sodium dichloroisocyanurate

Sodium dichloroisocyanurate (NaDCC) in powder form contains 60% available chlorine. Solutions prepared with NaDCC powder at 1.7 g/l and 8.5 g/l will contain 1 g/l or 5 g/l available chlorine, respectively. Tablets of NaDCC generally contain the equivalent of 1.5 g available chlorine per tablet. One or four tablets dissolved in 1 liter of water will give approximately the required concentrations of 1 g/l or 5 g/l, respectively. NaDCC as powder or tablets is easy and safe to store. Solid NaDCC can be applied on spills of blood or other biohazardous liquids for at least 10 min before removal. Further cleaning of the affected area can then take place.

Chloramines

Chloramines are available as powders containing about 25% available chlorine. Chloramines release chlorine at a slower rate than hypochlorites. Higher initial

concentrations are therefore required for efficiencies equivalent to those of hypochlorites. On the other hand, chloramine solutions are not inactivated by organic matter to the same extent as hypochlorite solutions, and concentrations of 20 g/l are recommended for both “clean” and “dirty” situations.

Chloramine solutions are virtually odor-free. However, items soaked in them must be thoroughly rinsed to remove any residue of the bulking agents added to chloramine T (sodium tosylchloramide) powders.

Chlorine dioxide

Chlorine dioxide (ClO_2) is a powerful, fast-acting germicide, disinfectant agent and oxidizer, often reported to be active at concentrations levels lower than those needed by chlorine bleach. Chlorine dioxide is unstable as a gas and will undergo decomposition into chlorine gas (Cl_2) and oxygen gas (O_2), giving off heat. However, chlorine dioxide is soluble in water and stable in aqueous solution. Chlorine dioxide can be obtained in two ways: (1) on-site generation by mixing two separate components, hydrochloric acid (HCl) and sodium chlorite ($NaClO_2$); and (2) ordering its stabilized form, which is then activated on-site when required.

Of the oxidizing biocides, chlorine dioxide is the most selective oxidant. Ozone and chlorine are much more reactive than chlorine dioxide and they will be consumed by most organic compounds. Chlorine dioxide, however, reacts only with reduced sulfur compounds, secondary and tertiary amines and some other highly reduced and reactive organic compounds. A more stable residue can therefore be achieved with chlorine dioxide at much lower concentrations than when using either chlorine or ozone. Generated properly, chlorine dioxide can be used more effectively than ozone or chlorine when there is higher organic loading because of its selectivity.

Formaldehyde

Formaldehyde (HCHO) is a gas that kills all microorganisms and spores at temperatures above 20°C. However, it is not active against prions.

Formaldehyde is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, paraformaldehyde, in flakes or tablets, or as formalin, a solution of formaldehyde gas in water of about 370 g/l (37%), containing methanol (100 ml/l) as a stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as biosafety cabinets and rooms (see section on Local environmental decontamination in this chapter). Formaldehyde (5% formalin in water) may be used as a liquid disinfectant, but not in open containers or surfaces.

Formaldehyde is a suspected carcinogen. It is a dangerous irritant gas that has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a chemical fumehood, sealed spaces or in well-ventilated areas.

Glutaraldehyde

Like formaldehyde, glutaraldehyde ($OHC(CH_2)_3CHO$) is also active against vegetative bacteria, spores, fungi and lipid- and non-lipid-containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill some microbial spores.

Glutaraldehyde is generally supplied as a solution with a concentration of about 20 g/l (2%) and some products may need to be “activated” (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation and type and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid.

Glutaraldehyde is toxic and an irritant to skin and mucous membranes. Contact with it must be avoided. It must be used in a chemical fumehood or in well-ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces. National chemical safety regulations must be followed.

Phenolic compounds

Phenolic compounds, a broad group of agents, were among the earliest germicides. However, more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against non-lipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (e.g. Triclosan™ and Chloroxylenol™) are among the more commonly used antiseptics.

Triclosan™ is common in products for hand-washing, but it’s use is not recommended. It is active mainly against vegetative bacteria. However, laboratorybased studies show that bacteria that have become resistant to low concentrations of Triclosan™ also show resistance to certain types of antibiotics.

Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water.

Phenolic compounds are not recommended for use on food contact surfaces and in areas with young children. They may be absorbed by rubber and can also penetrate the skin

Quaternary ammonium compounds

Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against some vegetative bacteria and lipid-containing viruses. Certain types (e.g. benzalkonium chloride) are used as antiseptics.

The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness and anionic detergents. Care is therefore needed when selecting agents for pre-cleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Because of their low biodegradability, these compounds may also accumulate in the environment.

Alcohols

Ethanol (ethyl alcohol, C_2H_5OH) and 2-propanol (isopropyl alcohol, $(CH_3)_2CHOH$) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses, but not spores. Their action on non-lipid viruses is variable. For greatest effectiveness, they should be used at concentrations of approximately 70% (v/v) in water. Higher or lower concentrations have less germicidal activity. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. Alcohols are not effective disinfectants for operating biosafety cabinets because they evaporate within 15 seconds.

Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol with 100 g/l formaldehyde and alcohol containing 2 g/l available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and for soaking small surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in healthcare situations where proper hand-washing with running water is inconvenient or not possible. However, it must be remembered that ethanol is not effective against spores and may not inactivate non-lipid viruses.

Alcohols are volatile and flammable and must not be used near open flames. Workingsolutions should be stored in proper containers to avoid the evaporation of alcohols.Alcohols may harden rubber, damage plastics and dissolve certain types of glue. Proper inventory andstorage of ethanol in the laboratory is very important. Itsuse for purposesother than disinfection is removal of grease and lipids. Bottles with alcohol-containing solutions must be clearlylabeled to avoid autoclaving.

Iodophors and Iodine

The action of these disinfectants is similar to that of chlorine, although they may beslightly less inhibited by organic matter. Elemental iodine is generally unsuitable for use as a disinfectant.Iodine should not be used on aluminum or copper.

Iodophors, such as Wescodyne^{TM},make excellent disinfectants and are commonly used on surfaces such as biosafety cabinet work surfaces and in water baths to reduce growth of microorganisms. They leave a brown residue on environmental surfaces that can be easily removed. If the residue is left on biosafety cabinet work surfaces, the operator can see where liquids drop on the work surface (leaving a clear circle).

Iodophors and tinctures of iodine are good antiseptics. Povidone-iodine is a reliableand safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodineare generally not used on medical/dental devices.

Hydrogen peroxide and peracids

Like chlorine, hydrogen peroxide (H_2O_2) and peracids are strong oxidants and can bepotent broad-spectrum germicides. They are also safer than chlorine to humans andthe environment.

Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30%aqueous solution to be diluted to 5–10 times its volume with sterilized water. However,such 3–6% solutions of hydrogen peroxide alone are relatively slow acting and limited asgermicides. Products now available have other ingredients to stabilize the hydrogenperoxide, accelerate its germicidal action and make it less corrosive.

Hydrogen peroxide can be used for the decontamination of work surfaces oflaboratory benches and biosafety cabinets. More concentrated solutions may be suitable fordisinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogenperoxide or peracetic acid (CH_3CO_3H) for the decontamination of heat-sensitivemedical/surgical devices requires specialized equipment.

Hydrogen peroxide and peracids can be corrosive to metals such as aluminum,copper, brass and zinc and can also decolorize fabrics, hair, skin and mucousmembranes. Articles treated with them must be thoroughly rinsed before contact witheyes and mucous membranes. They should be stored away from heat andprotected from light.

Local environmental decontamination

Decontamination of laboratory spaces, furniture and equipment requires acombination of liquid and gaseous disinfectants. Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl). A solution containing 1 g/l available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/l) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide (H_2O_2) make suitable substitutes for bleach solutions.

Rooms and equipment can be decontaminated with formaldehyde gas generated by heating paraformaldehyde or boiling formalin. This is a dangerous process that requires specially trained personnel. All openings in the room (i.e. windows, doors, etc.) must be sealed with duct tape or similar before the gas is generated. Gas decontamination should be conducted at ambient temperature and a relative humidity of 65-80%. (See also section on Decontamination of biosafety cabinets in this chapter.)

After space decontamination, the area must be ventilated thoroughly before personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Gaseous ammonium bicarbonate is used to neutralize the formaldehyde gas.

Spaces and equipment, such as biosafety cabinets, can also be decontaminated with chlorine dioxide gas or hydrogen peroxide vapor. Special equipment is used to generate the chlorine dioxide gas or hydrogen peroxide vapor. A space decontamination professional should be contacted.

Decontamination of biosafety cabinets

To decontaminate biosafety cabinets, equipment that independently generates, circulates and neutralizes formaldehyde gas is available. This is the procedure described in NSF/ANSI 49-2014 (NSF International, Ann Arbor, MI, USA). Multiply the total volume of the cabinet by 0.30 g/ft^3 (11 g/m^3) of space to determine the gram weight of paraformaldehyde required. The appropriate amount of paraformaldehyde (final concentration of 0.8% paraformaldehyde in air) should be placed in a frying pan on an electric hot plate. Another frying pan, containing 10% greater (by weight) ammonium bicarbonate than paraformaldehyde, is also placed inside the cabinet. The hot plate leads are plugged in outside the cabinet, so that operation of the pans can be controlled from the outside by plugging and unplugging the hot plates as necessary. If the relative humidity is below 65%, an open container of hot water should also be placed inside the cabinet before the front opening and exhaust port is sealed with heavy gauge plastic and strong tape (e.g. duct tape)to prevent formaldehyde gas escape into the room. Penetration of the electric leads passing through the front closure must also be sealed with duct tape.

The plate for the paraformaldehyde pan is plugged in. After 25% of the paraformaldehyde has depolymerized, turn on the cabinet blower(s) for 10 to 15 s. Repeat after 50%, 75%, and 100% of the paraformaldehyde has depolymerized. In cases where the cabinet blower is inoperative, circulation of air within the cabinet should be promoted with additional blowers or fans, or the time of decontamination should be extended beyond the times suggested. The paraformaldehyde plate is unplugged when all the paraformaldehyde has vaporized. The cabinet is left undisturbed for at least 6 hours, preferably overnight. The second hot plate is then plugged in and the ammonium bicarbonate is allowed to vaporize. As with the paraformaldehyde, after 25% of the NH_4HCO_3 has depolymerized, turn on the cabinet blower(s) for 10 to 15 s. In cases where the cabinet blower is inoperative, circulation of air within the cabinet should be promoted with additional blowers or fans, or the time of neutralization should be extended to a minimum of 6 h. This plate is then unplugged and the cabinet is allowed to stand at least an hour before opening seals. If a flexible hose has been provided for the evacuation of the neutralized formaldehyde, slit the plastic covering the BSC exhaust opening and seal the flexible hose to the opening. If the hose is working properly, the plastic covering the front opening of the cabinet should be sucked in. One or two small openings (approximately 6 x 6 in [15 x 15 cm]) are cut into the plastic covering the front opening of the cabinet to allow fresh air to enter the cabinet while the neutralized formaldehyde is being drawn out of the hose at the exhaust opening of the cabinet. Cabinet surfaces should be wiped down to remove residues before use.

Hand-washing/hand decontamination

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, after removing personnel protective equipment and before leaving the laboratory.

In most situations, thorough washing of hands with mild, non-antimicrobial soap and warm water for 30 seconds is sufficient. Use of germicidal soaps are only recommended for special situations. Hands should be thoroughly lathered with soap, using friction, for at least 30 seconds, rinsed in warm water and dried using a clean paper or cloth towel. If available, warm-air hand-dryers should be used.

Hands-free (with the electrical transformer located under the sink), foot- or elbow-operated faucets are recommended. A paper/cloth towel should be used to turn off standard faucet handles to avoid contaminating washed hands.

As mentioned above, alcohol-based hand-rubs are only used in healthcare settings to decontaminate lightly soiled hands when proper hand-washing is not available.

Heat disinfection and sterilization

Heat is the most common physical agent used for the decontamination of pathogens. “Dry” heat, which is non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160°C or higher for 2–4 h. Burning or incineration (see below) is also a form of dry heat. “Moist” heat is most effective when used in a pressure cooker (autoclave).

Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination, autoclaving) are not applicable or are not available.

Sterilized items must be handled and stored so that they remain uncontaminated until use.

Autoclaving

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials.

For sterilization of non-laboratory waste, the following minimum cycle times may ensure sterilization of correctly loaded autoclaves:

1. 4 min holding time at 134°C (pre-vacuum autoclave)
2. 15 min holding time at 121°C (gravity displacement autoclave)

For decontamination of laboratory waste, the following minimum cycle timesmay ensure decontamination of correctly loaded autoclaves:

1. 20-40 min holding time at 134°C (pre-vacuum autoclave)
2. 60-120 min holding time at 121°C (gravity displacement autoclave)

Examples of different autoclaves include the following:

Gravity displacement autoclaves. Figure 13 shows the general construction of a gravity displacement autoclave. Steam enters the chamber under pressure at the upper rear and displaces the heavier air downwards and through a drain at the bottom front of the chamber.At the end of the cycle, the steam is automatically exhausted slowly for liquids or quickly for non-liquids. These autoclaves operate at 121°C and the sterilization cycle for clean materials may be as short as 15 minutes. The cycle for decontamination of laboratory waste is as short as 60-120 minutes. The overkill method (double time) is often used for decontamination – destruction or inactivation of microorganisms with the probability of one in a trillion surviving the process.

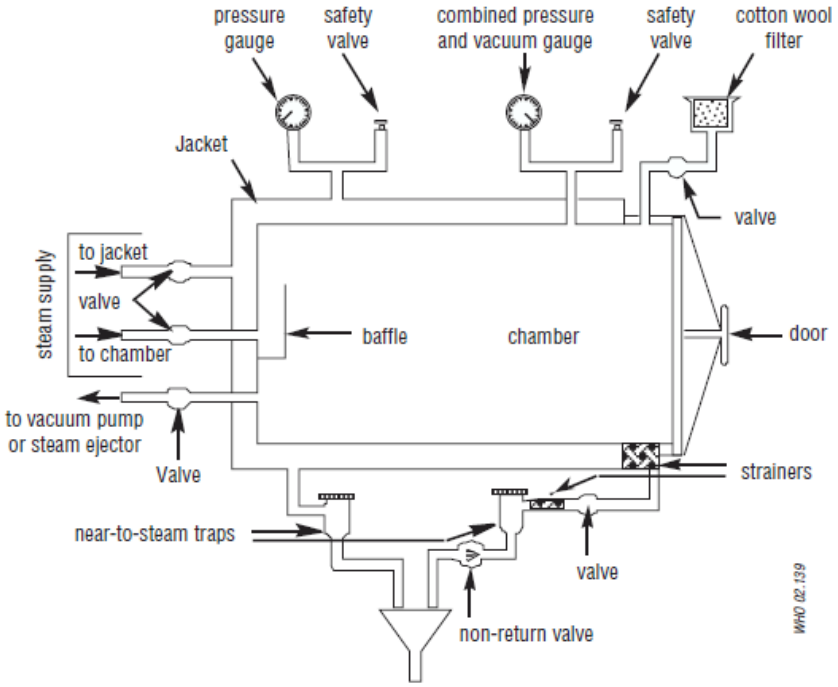


Figure 13. Gravity displacement autoclave

Pre-vacuum autoclaves. These autoclaves remove air from the chamber before steam is admitted. At the end of the cycle, the steam is automatically exhaustedslowly for liquids or quickly for non-liquids. These autoclaves operate at 134°C and the sterilization cycle of clean materials can be reduced to as short as 4 minutes. The cycle for decontamination of laboratory waste is as short as 20-40 minutes. They are ideal for porous loads, but containers of liquid must be open during the cycle, sometimes causing loss of liquid, because of the vacuum produced before steam enters the chamber.

Fuel-heated pressure cooker autoclaves. These should be used only if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas, electricity or other types of fuels. Steam is generated by heating water in the base of the vessel and air is displaced upwards through a relief vent. When all the air has been removed, the valve on the relief vent is closed and the heat is reduced. The pressure and temperature rise until the safety valve operates at a preset level. This is the start of the holding time. At the end of the cycle the heat is turned off and the temperature allowed to fall to 80°C or below before the lid is opened.

Flash sterilization autoclaves. These autoclaves are used for the sterilization of critical medical devices that have become contaminated during surgical procedures and must be returned to the sterile field. Flash sterilization is not recommended as a routine sterilization method because of the lack of timely biological indicators to monitor

performance. Flash sterilizers are modified conventional autoclaves. Items to be flashed sterilized are placed in an open tray or in a specially designed, covered, rigid container to allow for rapid penetration of steam. Flash sterilization of unwrapped objects usually takes 4 minutes at 132-134°C, but the time required for flash sterilization depends on the type of sterilizer and the type of item (i.e., porous vs non-porous items). Flash sterilizers are usually placed in close proximity to operating rooms to facilitate aseptic delivery to the point of use.

Loading autoclaves. Materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should be open to allow the steam to reach their contents.

Precautions for use of autoclaves. The following rules can minimize the hazards inherent with operation of pressurized vessels.

1. Responsibility for operation and routine care should be assigned to trained individuals.
2. A preventive maintenance program should include regular inspection of the chamber, door seals and all gauges and controls by qualified personnel.
3. The steam should be saturated and free from chemicals (e.g. corrosion inhibitors) that could contaminate the items being sterilized.
4. All materials to be autoclaved should be in containers that allow easy removal of air and permit good heat penetration; the chamber should be loosely packed so that steam will reach the load evenly.
5. For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80°C before the door is opened.
6. Slow exhaust settings should be used when autoclaving liquids, because they may boil over when removed due to superheating.
7. Operators should wear suitable gloves and visors for protection when opening the autoclave, even after the temperature has fallen below 80°C.
8. For routine monitoring of autoclave performance, biological indicators or thermocouples should be placed at the center of each load. Regular monitoring with thermocouples and recording devices in a “worst case” load is highly desirable to determine proper operating cycles.
9. The drain screen filter of the chamber (when present) should be removed and cleaned daily.
10. Care should be taken to ensure that the relief valves of pressure cooker autoclaves do not become blocked by paper, etc. in the load.

Incineration

Incineration is useful for disposal of animal carcasses as well as anatomical and other laboratory waste, with or without prior decontamination (see Chapter 2). Incineration of infectious materials is an alternative to autoclaving only if the incinerator is controlled by the laboratory.

Proper incineration requires an efficient means of temperature control and a secondary burning chamber. Many incinerators, especially those with a single combustion chamber, are unsatisfactory for dealing with infectious materials, animal carcasses and plastics. Such materials may not be completely destroyed and the gas effluent from the chimney may pollute the atmosphere with microorganisms, toxic chemicals and smoke. However, there are many satisfactory configurations for combustion chambers. Ideally the temperature in the primary chamber should be at least 800°C and at least 1000°C in the secondary chamber.

Materials for incineration, even with prior decontamination, should be transported to the incinerator in plastic bags or preferably plastic-lined boxes or hand trucks. Incinerator attendants should receive proper instructions for loading and temperature control. Efficient operation of an incinerator depends heavily on the right mix of materials in the waste being treated.

Superheated water grinding

Superheated water grinding effectively sterilizes and grinds potentially infectious medical waste including small anatomical waste, cultures, glass, plastics, needles (including 26 gauge 10 mm tuberculin needles), gloves, autoclave bags, etc. into small solids that are no longer recognizable as medical waste. The dry solids are acceptable as municipal waste. There are no air emissions and liquid effluent is acceptable to municipal sewage treatment facilities. The efficacy of this system for safe disposal of infectious medical waste is more cost-effective than other technologies.

Alkaline hydrolysis

Alkaline hydrolysis is an optimum method for disposal of anatomic and pathologic waste generated in healthcare and medical and veterinary education; animal tissue and carcasses from biomedical and pharmaceutical research facilities; and government research and diagnostic facilities. Alkaline hydrolysis uses water solutions of alkali metal hydroxides such as sodium hydroxide (NaOH) or potassium hydroxide (KOH). Heating the reactants dramatically accelerates the hydrolysis reaction. The alkali liquid is neutralized before the liquid effluent is sent to municipal sewage. This process is also an alternative to cremation

Disposal

Disposal of laboratory and medical waste is subject to various regional, national and international regulations. The latest versions of such relevant documents must be consulted before designing and implementing a program for handling, transportation and disposal of biohazardous waste. In general, ash from incinerators may be handled as normal domestic waste and removed by local authorities. Autoclaved waste may be disposed by off-site incineration or in licensed landfill sites (see Chapter 2).

For further information, see references (13 and 29–39).

14. Introduction to the transport of infectious substances

Transport of infectious and potentially infectious materials is subject to strict national and international regulations. These regulations describe the proper use of packaging materials, as well as other shipping requirements.

Laboratory personnel must ship infectious substances according to applicable transport regulations. Compliance with the rules will:

1. Reduce the likelihood that packages will be damaged and leak.
2. Reduce the exposures resulting in possible infections.
3. Improve the efficiency of package delivery.

International transport regulations

The regulations for the transport of infectious materials (by any mode of transport) are based upon the United Nations Model Regulations on the Transport of Dangerous Goods (40). These recommendations are developed by the United Nations Subcommittee of Experts on the Transport of Dangerous Goods (SCoETDG)

The International Air Transport Association (IATA) issues Dangerous Goods Regulations (DGR)(43) every year. The DGR include regulations for shipping infectious substances by air. IATA guidelines follow the International Civil Aviation Organization (ICAO) Technical Instructions for the SafeTransport of Dangerous Goods by Air (41). Individual states and air carriers may impose additional restrictions. IATA guidelines must be followed if a shipment is carried by members of IATA.

Since the United Nations Model Regulations on the Transport of Dangerous Goods is a dynamic set of recommendations subject to amendments, the reader is referred to the latest issue of national and international modal regulations for applicable regulatory texts.

Major changes to the transport regulations pertaining to the transport of infectious substances were introduced into the 13th edition (2003) of the United Nations Model Regulations (40). Guidance on the background to adopted amendments is available from WHO (44).

It is important to note that international transport of infectious substances is also dependent on and subject toQatar customs and security guidelines.

Classes of Dangerous Goods

("Hazardous Materials" term is only used by the United States)

Class 1	Explosives
Class 2	Flammable, Non-flammable, or Toxic Gases
Class 3	Flammable Liquid
Class 4	Flammable Solids, Spontaneously Combustible when Wet
Class 5	Oxidizer or Organic Peroxide
Class 6	Toxic or Infectious Substance
Class 7	Radioactive Material
Class 8	Corrosive
Class 9	Miscellaneous Dangerous Goods, such as Dry Ice, solid

Class 6, Division 6.1 Toxic Substances

Toxins extracted from living sources with no infectious substances are UN 3172.

Packing instructions vary with oral toxicity, dermal toxicity or inhalation criteria for dusts and mists.

Class 6, Division 6.2 Infectious Substances

Infectious substances are known to contain, or reasonably expected to contain pathogens. Infectious substances must be classified in Division 6.2 and assigned to UN 2814, UN 2900, UN 3291 or UN 3373, as appropriate

Pathogens are microorganisms (bacteria, viruses, rickettsia, parasites and fungi, or prions) which can cause disease in humans or animals.

Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development,

experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines.

Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include patient specimens.

Patient specimens those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Medical or clinical wastes are wastes derived from the medical treatment of animals or humans or from biological research.

The basic triple packaging system

The triple packaging systemfor transport of Category A Infectious Substances is shown in Figure 14. The triple packaging systemfor the transport of Category B Biological Substances is shown in Figure 15. These packaging systems consist of three layers: the primary receptacle, the secondary packaging and the outer packaging.

The primary receptacle containing the specimen must be watertight, leak proof and appropriately labelled as to content. The primary receptacle is wrapped in enoughabsorbent material to absorb all fluid in case of breakage or leakage.

A second watertight, leak proof packaging is used to enclose and protect the primaryreceptacle(s). Several wrapped primary receptacles may be placed in a single secondarypackaging. Volume and/or weight limits for packaged infectious substances are includedin regulatory texts.

The third layer, usually a certified box, protects the secondary packaging from physical damage while intransit. Specimen data forms, letters and other types of information that identify ordescribe the specimen, identify the shipper and receiver and any otherrequired documentation must also be provided according to current regulations.

The United Nations Model Regulations prescribe the use of three different triplepackaging systems for infectious substances, biologic substances and patient specimens, respectively. The basic triple packaging system applies to the transport of avariety of materials. However, Category A Infectious Substances must be shippedaccording to more stringent requirements. For further details about the use of specific packaging according to the materials to be transported, it is advisable toconsult Qatar customs and security guidelines.

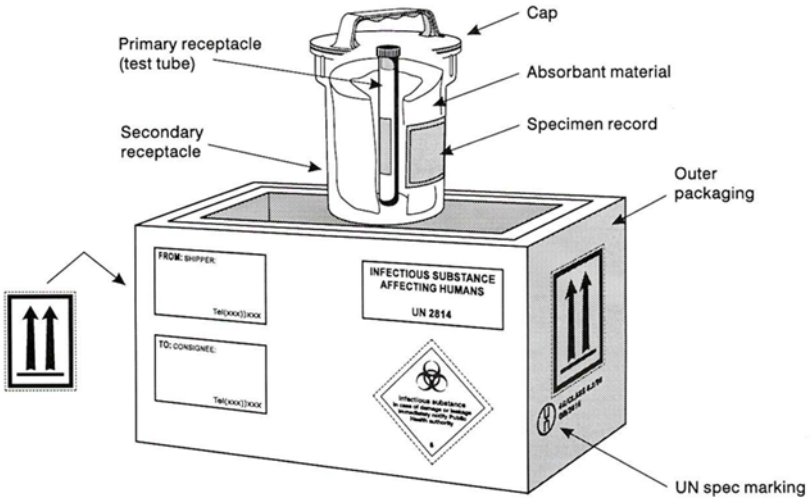


Figure 14. Packing and labeling of Category A Infectious Substances

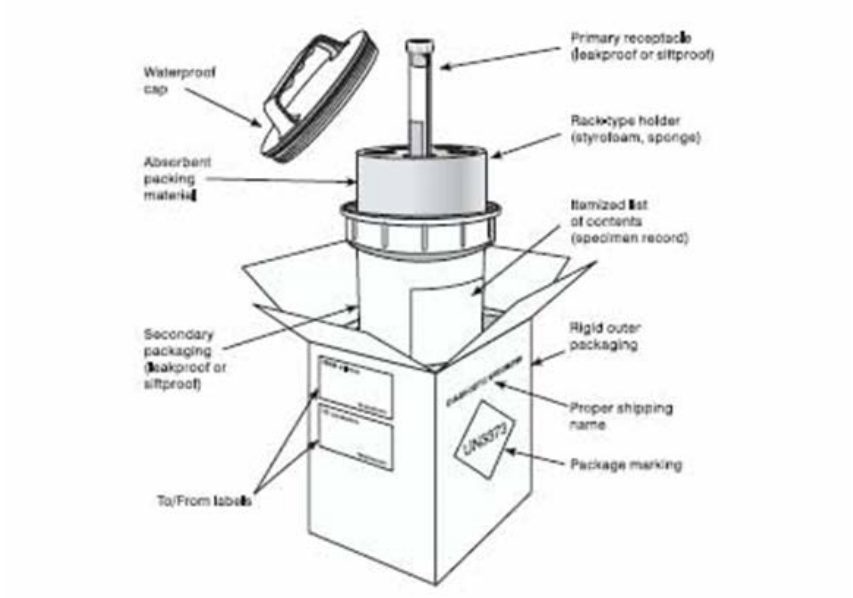


Figure 15. Packing and labeling of Category B Biological Substances

Spill clean-up procedure

In the event of a spill of infectious or potentially infectious material, the followingspill clean-up procedure should be used.

1. Wear gloves and protective clothing, including face and eye protection if indicated.
2. Cover the spill with cloth or paper towels to contain it.
3. Pour an appropriate disinfectant over the paper towels and the immediatesurrounding area (generally, 1:10 diluted bleach solution is appropriate; but for spills onaircraft, quaternary ammonium disinfectants should be used).
4. Apply disinfectant concentrically beginning at the outer margin of the spill area,working toward the center.
5. After the appropriate amount of time (e.g. 10-30 minutes), clear away the materials. Ifthere is broken glass or other sharps involved, use a dustpan, forceps or a piece of stiffcardboard to collect the material and deposit it into a puncture-resistant containerfor disposal.
6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials into a leak-proof, puncture-resistant waste disposal container.
8. After successful disinfection, inform the MOPH Research Department that the site has now been decontaminated

PART V

Introduction to biotechnology

15. Biosafety and recombinant or synthetic nucleic acid technology

Recombinant or synthetic nucleic acid technology involves combining genetic material from different sources thereby creating genetically modified organisms (GMOs) that may have never existed in nature before. Initially there was concern among molecular biologists that such organisms might have unpredictable and undesirable properties that could represent a biohazard if they escaped from the laboratory. This concern became the focus of a scientific conference held in Asilomar, CA, USA, in 1975 (47). At that meeting, safety issues were discussed and the first guidelines for recombinant DNA technology were proposed. The subsequent 25+ years of research experience have demonstrated that genetic engineering may be conducted in a safe manner when an appropriate risk assessment is performed and adequate safety measures are used.

Recombinant or synthetic nucleic acid technology has already had an enormous impact on biology and medicine and will probably have an even greater influence now that the nucleotide sequence of the entire human genome is available. Tens of thousands of genes of yet unknown functions will be studied using recombinant or synthetic nucleic acid technology. Gene therapy may become a routine treatment for certain diseases and new vectors for gene transfer are likely to be devised using genetic engineering techniques. Also, transgenic plants produced by recombinant or synthetic nucleic acid technology may play an increasingly important role in modern agriculture.

Experiments involving the construction or use of GMOs should be conducted afterperforming a biosafety risk assessment. The pathogenic properties and any potentialhazards associated with such organisms may be novel and not well-characterized. Theproperties of the donor organism, the nature of the nucleic acid sequences that will betransferred, the properties of the recipient organism and the effects on theenvironment should be evaluated. These factors should help determine the biosafetylevel that is required for the safe handling of the resulting GMO and identify thebiological and physical containment systems that should be used.

Biosafety considerations for biological expression systems

Biological expression systems consist of vectors and host cells. A number of criteria must be satisfied to make them effective and safe to use. An example of a biological expression system is plasmid pUC18. Frequently used as a cloning vector in combination with Escherichia coli K12 cells, the pUC18 plasmid has been entirely sequenced. All genes

required for expression in other bacteria have been deleted from its precursor plasmid pBR322. E. coli K12 is a non-pathogenic strain that cannot permanently colonize the gut of healthy humans or animals. Routine genetic engineering experiments can safely be performed in E. coli K12/pUC18 at Biosafety Level 1, provided the inserted foreign DNA expression products do not require higher biosafety levels.

Biosafety considerations for expression vectors

Higher biosafety levels may be required when:

1. The expression of nucleic acid sequences derived from pathogenic organisms may increase the virulence of the GMO.
2. Inserted nucleic acid sequences are not well characterized, e.g. during preparation of genomic DNA libraries from pathogenic microorganisms.
3. Gene products have potential pharmacological activity.
4. Gene products code for toxins.

Viral vectors for gene transfer

Viral vectors, e.g. adenovirus vectors, are used for the transfer of genes to other cells. Such vectors lack certain virus replication genes and are propagated in cell lines that complement the defect.

Stocks of such vectors may be contaminated with replication-competent viruses generated by rare spontaneous recombination events in the propagating cell lines or may appear after insufficient purification. These vectors should be handled at the same biosafety level as the parent virus from which they are derived.

Transgenic and “knock-out” animals

Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes (“knock-out” animals) do not generally present particular biological hazards.

Examples of transgenic animals include animals expressing receptors for viruses normally unable to infect that species. If such animals escape from the laboratory and transmit their transgene to the wild animal population, an animal reservoir for that particular virus could theoretically be generated.

This possibility has been discussed for poliovirus and is particularly relevant in the context of poliomyelitis eradication. Transgenic mice expressing the human poliovirus receptor

generated in different laboratories were susceptible to poliovirus infection by various inoculation routes. The resulting disease was clinically and histopathologically similar to human poliomyelitis. However, the mouse model differs from humans because alimentary tract replication of orally administered poliovirus in the mouse is either inefficient or does not occur. It is therefore unlikely that escape of such transgenic mice to the wild would result in the establishment of a new animal reservoir for poliovirus. Nevertheless, this example indicates that for each new line of transgenic animal, detailed studies should be conducted to determine the routes by which the animals can be infected, the inoculum size required for infection and the extent of virus shedding by the infected animals. In addition, all measures should be taken to assure strict containment of receptor transgenic mice.

Transgenic plants

Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in many parts of the world. The discussions focus on the food-safety of such plants and on the long-term ecological consequences of their cultivation.

Transgenic plants expressing genes of animal or human origin are used to develop medicinal and nutritional products. A risk assessment should determine the appropriate biosafety level for the production of these plants.

Risk assessments for genetically modified organisms

Risk assessments for work with GMOs should consider the characteristics of donor and recipient/host organisms.

Examples of characteristics for consideration include the following.

Hazards arising directly from the inserted gene (donor organism)

Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example:

1. Toxins.
2. Cytokines.
3. Hormones.
4. Gene expression regulators.
5. Virulence factors or enhancers.
6. Oncogenic gene sequences.
7. Antibiotic resistance.
8. Allergens.

The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity.

Hazards associated with the recipient/host

1. Susceptibility of the host.
2. Pathogenicity of the host strain, including virulence, infectivity and toxin production.
3. Modification of the host range.
4. Recipient immune status.
5. Consequences of exposure.

Hazards arising from the alteration of existing pathogenic traits

Many modifications do not involve genes whose products are inherently harmful, butadverse effects may arise as the result of altering existing non-pathogenic orpathogenic traits. Modification of normal genes may alter pathogenicity. In an attemptto identify these potential hazards, the following points may be considered (the list isnot exhaustive).

1. Is there an increase in infectivity or pathogenicity?
2. Could any disabling mutation within the recipient be overcome as a result of theinsertion of the foreign gene?
3. Does the foreign gene encode a pathogenicity determinant from another organism?
4. If the foreign nucleic acid does include a pathogenicity determinant, is it foreseeable thatthis gene could contribute to the pathogenicity of the GMO?
5. Is treatment available?
6. Will the susceptibility of the GMO to antibiotics or other forms of therapy beaffected as a consequence of the genetic modification?
7. Is eradication of the GMO achievable?

For further information, see references (17 and 46-48).

PART VI

Chemical, fire andelectrical safety

16. Hazardous chemicals

Workers in microbiological laboratories are not just exposed to pathogenic microorganisms, but also to chemical hazards. It is important that they have proper knowledge of the toxic effects of these chemicals, the routes of exposure and the hazards that may be associated with their handling and storage (see Annex 5). Safety data sheets or other chemical hazard information are available from chemical manufacturers and/or suppliers. These should be accessible in laboratories where these chemicals are used, e.g. as part of a safety or operations manual.

Routes of exposure

Exposure to hazardous chemicals may occur by:

1. Inhalation.
2. Contact.
3. Ingestion.
4. Needle-sticks.
5. Through broken or unbroken skin.

Storage of chemicals

Only amounts of chemicals necessary for daily use should be stored in the laboratory.

Bulk stocks should be kept in specially designated chemical storage rooms or buildings.

Chemicals should not be stored in alphabetical order.

General rules regarding chemical incompatibilities

To avoid fire and/or explosions, substances in the left-hand column of Table 11 should be stored and handled so they will not come into contact with the corresponding substances in the right-hand column of the table.

Toxic effects of chemicals

Some chemicals adversely affect the health of those who handle them or inhale their vapors. Apart from overt poisons, a number of chemicals are known to have various toxic effects. The respiratory system, blood, lungs, liver, kidneys and the gastrointestinal

system, as well as other organs and tissues, may be adversely affected or seriously damaged. Some chemicals are known to be carcinogenic or teratogenic.

Table 11. General rules for chemical incompatibilities

SUBSTANCE CATEGORY	INCOMPATIBLE SUBSTANCES
Alkali metals, e.g. sodium, potassium, cesium and lithium	Carbon dioxide, chlorinated hydrocarbons, water
Halogens	Ammonia, acetylene, hydrocarbons
Acetic acid, hydrogen sulfide, aniline,hydrocarbons, sulfuric acid	Oxidizing agents, e.g. chromic acid, nitric acid,peroxides, permanganates

Some solvent vapors are toxic when inhaled. Apart from the more serious effects noted above, exposure may result in impairments that show no immediate discernible effects on health, such as lack of coordination, drowsiness and similar symptoms, leading to an increased proneness to accidents.

Prolonged or repeated exposures to the liquid phase of many organic solvents can result in skin damage. This may be due to a defatting effect, but allergic and corrosive symptoms may also arise.

Refer to these references for more information:

GHS (Rev.6) (2015) - Globally Harmonized System of Classification and Labelling of Chemicals (GHS) - Sixth revised edition - Copyright © United Nations, 2015
http://www.unece.org/trans/danger/publi/ghs/ghs_rev06/06files_e.html#c38156
http://www.unece.org/trans/danger/publi/ghs/pictograms.html

For detailed information on the toxic effects of chemicals see Annex 5.

Explosive chemicals

Azides, often used in antibacterial solutions, should not be allowed to come into contactwith copper or lead (e.g. in waste pipes and plumbing) or be allowed to dry because they may explode violentlywhen subjected to even a mild impact.

Ethers that have aged and dried to crystals are extremely unstable and potentiallyexplosive.

Perchloric acid, if allowed to dry on woodwork, brickwork or fabric, will explodeand cause a fire on impact.

Picric acid and picrates are detonated by heat and impact.

Chemical spills

Most manufacturers of laboratory chemicals issue charts describing methods fordealing with spills. Spill charts and spill kits are also available commercially.Appropriate charts should be displayed in a prominent position in the laboratory. Thefollowing equipment should also be provided:

1. Chemical spill kits.
2. Protective clothing, e.g. heavy-duty rubber or plastic gloves, overshoes or rubber boots,respirators.
3. Scoops and dustpans.
4. Forceps for picking up broken glass.
5. Mops, cloths and paper towels.
6. Buckets.
7. Soda ash (sodium carbonate, Na_2CO_3) or sodium bicarbonate ($NaHCO_3$) for neutralizing acids and corrosive chemicals.
8. Sand (to cover alkali spills).
9. Non-flammable detergent.

The following actions should be taken in the event of a significant chemical spill:

1. Notify the appropriate safety officer.
2. Evacuate non-essential personnel from the area.
3. Attend to persons who may have been contaminated and use a safety drench shower if appropriate.
4. If the spilled material is flammable, extinguish all open flames if possible and call the fire department, turn off gas in the room and adjacent areas, open windows (if possible) and switch off electrical equipment that may spark.
5. Avoid breathing vapor from spilled material.
6. Establish exhaust ventilation if it is safe to do so.
7. Secure the necessary items (see above) to clean up the spill.

Compressed and liquefied gases

Information regarding storage of compressed and liquefied gases is given in Table 12.

Table 12. Storage of compressed and liquefied gases

CONTAINER	STORAGE INFORMATION
Compressed gas cylinders and liquefied gas containers (a,b)	• Should be securely fixed (e.g. chained) to the wall or a solid bench so that they are not inadvertently dislodged. • Must be transported with their caps in place and supported on trolleys. • Should be stored in bulk in an appropriate facility at some distance from the laboratory. This area should be locked and appropriately identified. • Should not be placed near radiators, open flames, other heat sources, sparking electrical equipment or in direct sunlight.
Small, single-use gas cylinders (a,b)	• Must not be incinerated

a The main high-pressure valve should be turned off when the equipment is not in use and when the roomis unoccupied.

b Rooms where flammable gas cylinders are used and/or stored should be identified by warning notices onthe doors.

For further information, see references (1 and 49–51) and Annex 5.

17. Additional laboratory hazards

Laboratory personnel may confront hazards posed by forms of energy including fire, electricity, radiation and noise. Basic information about each of these is presented in this chapter.

Fire hazards

Close cooperation between safety officers and civil defense is essential. Apart from chemical hazards, the effects of fire on the possible dissemination of infectious material must be considered. This may determine whether it is best to extinguish or contain the fire.

It is recommended that the assistance of civil defense officers be used when training laboratory staff about fire prevention, immediate actions when there is a fire and use of fire-fighting equipment.

Fire warnings, instructions and escape routes should be displayed prominently in each room and in corridors and hallways.

Common causes of fires in laboratories are:

1. Electrical circuit overloading.
2. Poor electrical maintenance, e.g. poor and damaged insulation on cables.
3. Excessively long gas tubing or long electrical leads.
4. Equipment unnecessarily left turned on.
5. Equipment that was not designed for a laboratory environment.
6. Open flames.
7. Deteriorated gas tubing.
8. Improper handling and storage of flammable or explosive materials.
9. Improper segregation of incompatible chemicals.
10. Sparking equipment near flammable substances and vapors.
11. Improper or inadequate ventilation.

Fire-fighting equipment should be placed near room doors and at strategic points in corridors and hallways. This equipment may include hoses, buckets (of water or sand) and a fire extinguisher. Fire extinguishers should be regularly inspected and maintained. Their expiration date should be current. Specific types and uses of fire extinguishers are presented in Table 13.

Table 13. Types and uses of fire extinguishers

TYPE	USE FOR	DO NOT USE FOR
Water	Paper, wood, fabric	Electrical fires, flammable liquids, burning metals
Carbon dioxide (CO2)extinguisher	Flammable liquids and gaseselectrical fires	Alkali metals, paper
Dry powder	Flammable liquids and gases, alkali metals, electrical fires	Reusable equipment and instruments, as residues are very difficult to remove
Foam	Flammable liquids	Electrical fires

For further information, see reference (49).

Electrical hazards

All electrical installations and equipment must be inspected and tested regularly, including grounding systems.

Circuit-breakers and ground-fault-interrupters should be installed in appropriate laboratory electrical circuits. Circuit-breakers do not protect people; they are intended to protect wiring from being overloaded with electrical current and causing fires. Ground-fault-interrupters are intended to protect people from electric shock.

All laboratory electrical equipment should be grounded, preferably through three-prong plugs.

All laboratory electrical equipment and wiring should conform toelectrical safety standards and codes.

Noise

Excessive noise can cause hearing loss over time. Some types of laboratory equipmentand facilities where animals are housed can producesignificant noise exposure to workers. Noise measurement surveys can be conductedto find noise hazards. Whensurveys find noise hazards, engineering controls such asenclosures or barriers around noisy equipment or between noisy areas and other work areas can be considered. When noise levels cannot be abated and laboratorypersonnel routinely experience

excessive noise exposure, a hearing conservation program should be established that includes hearing protection for the workers and amedical monitoring program to determine the effect of noise on the workers'hearing.

Ionizing radiation

Radiological protection protectsworkers from the harmfuleffects of ionizing radiation, which include:

1. Somatic effects, e.g. clinical symptoms observable in exposed individuals. Somatic effects include radiation-induced cancers, (e.g. leukemia and bone, lung and skin cancers) that may not appear for many years after exposure. Less severe somatic effects include minor skin damage, hair loss, blood deficiencies, gastrointestinal damage and cataract formation.

2. Hereditary effects, e.g. symptoms observed in the descendants of exposed individuals. The hereditary effects of radiation exposure to the gonads include chromosome damage or gene mutation. Irradiation of the germ cells in the gonads in high doses can also cause cell death, resulting in impaired fertility in both sexes ormenstrual changes in women. Exposure of the developing fetus, particularly inweeks 8–15 of pregnancy, may increase the risk of congenital malformations, mentalimpairment or radiation-induced cancers in later life.

Principles of ionizing radiation protection

To limit the harmful effects of ionizing radiation, the use of radioisotopes should becontrolled and should comply with relevant national standards. Protection fromradiation is managed on the basis of four principles:

1. Minimizing the time of radiation exposure.
2. Maximizing the distance from the radiation sources.
3. Shielding radiation sources.
4. Substituting the use of radionuclides with non-radiometric techniques.

Protection activities include the following.

1. Time. The time of exposure experienced during manipulations of radioactivematerial can be reduced by:

- Practicing new and unfamiliar techniques without using the radionuclide untilthe techniques are mastered.
- Working with radionuclides in a deliberate and timely manner without rushing.

- Ensuring that all radioactive sources are returned to storage immediately afteruse.
- Removing radioactive waste from the laboratory at frequent intervals.
- Spending as little time as possible in the radiation area or laboratory.
- Exercising effective time management and planning of laboratory manipulationsinvolving radioactive material.

The less time spent in a radiation field the smaller the received personal dose, asdescribed in the equation:

Dose = Dose rate x time

2. *Distance*. The dose rate for most γ- and X-radiation varies as the inverse square ofthe distance from a point source:

Dose rate = Constant x 1/Distance^2

Doubling the distance from a radiation source will reduce exposure by one-fourth over the same period of time. Various devices and mechanical aids are used to increase the distance between the operator and the radiation source, e.g. long-handled tongs, forceps, clamps and remote pipetting aids. Note that a small increase in distance can result in significant decrease in the dose rate.

3. *Shielding*. Radiation energy-absorbing or attenuating shields placed between the source and the operator or other occupants of the laboratory will help limit their exposure. The choice and thickness of any shielding material depends on the penetrating ability (type and energy) of the radiation. A barrier of acrylic, wood or lightweight metal, thickness 1.3–1.5 cm, provides shielding against high-energy β particles, whereas high-density lead is needed to shield against high energy γ- and X-radiation.

4. *Substitution*. Radionuclide-based materials should not be used when other techniques are available. If substitution is not possible, then the radionuclide with the least penetrating power or energy should be used.

Safe practices for work with radionuclides

Rules for working with radioactive substances should include considerations in four areas:

1. Radiation area.
2. Work-bench area.
3. Radioactive waste area.
4. Records and emergency response.

Some of the most important rules include the following:

1. Radiation area

- Use radioactive substances only in dedicated areas.
- Allow the presence of essential staff only.
- Use personal protective equipment, including laboratory coats, safety glasses and disposable gloves.
- Monitor personal radiation exposures.

Laboratories where radionuclides are used should be designed to simplify containment, cleaning and decontamination. The radionuclide work area should be located in a small room adjoining the main laboratory or in a dedicated area within the laboratory away from other activities. Signs displaying the international radiation

hazard symbol should be posted at the entrance to the radiation area (Figure 16).

Figure 16. International radiation hazard symbol

2. Work-bench area

- Use spill trays lined with disposable absorbent materials.
- Limit radionuclide quantities.
- Shield radiation sources of the radiation, work bench and radioactive waste areas.
- Mark radiation containers with the radiation symbol, including radionuclide identity, activity and assay date.
- Use radiation meters to monitor working areas, protective clothing and hands after completion of work.
- Use appropriately shielded transport containers.

3. Radioactive waste area

- Remove radioactive waste frequently from the work area.
- Maintain accurate records of use and disposal of radioactive materials.
- Screen dosimetry records for materials exceeding the dose limits.
- Establish and regularly exercise emergency response plans.
- In emergencies, assist injured individuals first.

- Clean contaminated areas thoroughly.
- Request assistance from the safety office, if available.
- Write and keep incident reports.

PART VII

Safety organizationand training

18. The biosafety officer andbiosafety committee

It is essential that each laboratory organization have a comprehensive safety policy, a safety manual and supporting programs for their implementation. The responsibility for this normally rests with the director or head of the institute or laboratory who may delegate certain duties to a biosafety officer or other appropriate personnel.

Laboratory safety is also the responsibility of all supervisors and laboratory employees. Individual workers are responsible for their own safety and that of their colleagues. Employees are expected to perform their work safely and should report any unsafe acts, conditions or incidents to their supervisor. Periodic safety audits by internal or external personnel are desirable.

Biosafety officer

Wherever possible a biosafety officer should be appointed to ensure that biosafety policies and programs are followed consistently throughout the laboratory. The biosafety officer executes these duties on behalf of the head of the institute or laboratory. In small units, the biosafety officer may be a microbiologist or a member of the technical staff who may perform these duties on a defined part-time basis.

Whatever the degree of involvement in biosafety, the designated person should have the professional competence necessary to suggest, review and approve specific activities that follow appropriate biocontainment and biosafety procedures. The biosafety officer should apply relevant MOPH guidelines and international rules, regulations and guidelines, as well as assist the laboratory when developing standard operating procedures. The appointed person must have a technical background in microbiology, biochemistry and basic physical and biological sciences. Knowledge of laboratory and clinical practices and safety, including containment equipment and engineering principles relevant to the design, operation and maintenance of facilities is highly desirable. The biosafety officer should also be able to communicate effectively with administrative, technical and support personnel.

The activities of the biosafety officer should include the following:

1. Biosafety, biosecurity and technical compliance consultations.
2. Periodic internal biosafety audits of technical methods, procedures and protocols, biological agents, materials and equipment.

3. Discussions of biosafety protocol or procedure violations with the appropriate persons.
4. Verification that all staff have received appropriate biosafety training.
5. Provision of annual continuing biosafety education.
6. Investigating incidents involving possible escape of potentially infectious or toxic material and reporting findings and recommendations to the laboratory director and biosafety committee.
7. Coordination with medical staff regarding possible laboratory-acquired infections.
8. Ensuring appropriate decontamination following spills or other incidents involving infectious material(s).
9. Ensuring proper waste management.
10. Ensuring appropriate decontamination of any apparatus or equipment prior to repair or servicing.
11. Maintaining awareness of community attitudes regarding health and environmental considerations.
12. Establishment of appropriate procedures for import/export of pathogenic material to/from the laboratory according to national regulations.
13. Reviewing the biosafety aspects of all plans, protocols and operating procedures for research work involving infectious agents prior to the implementation of these activities.
14. Establishment of a system to deal with emergencies.

Biosafety committee

A biosafety committee should be constituted to develop institutional biosafety policies and codes of practice. The biosafety committee should also review research protocols for work involving infectious agents, animal use, recombinant or synthetic nucleic acid and genetically modified materials. Other functions of the committee may include risk assessments, formulation of new safety policies and arbitration of disputes over safety issues.

The membership of the biosafety committee should reflect the diverse occupational areas of the organization as well as its scientific expertise. The composition of a basic biosafety committee may include:

1. Biosafety officer(s).
2. Scientists.
3. Medical personnel.
4. Veterinarian(s) (if work with animals is conducted).
5. Horticulturist(s) (if work with plants is conducted).
6. Representatives of technical staff.
7. Representatives of laboratory management.

The biosafety committee should seek advice from different departmental and specialist safety officers (e.g. with expertise in radiation protection, industrial safety, fire prevention, etc.) and may at times require assistance from independent experts in various associated fields, local authorities and national regulatory bodies. Community members may also be helpful if there is a particularly contentious or sensitive protocol under discussion.

19. Safety for support staff

The safe and optimum operation of a laboratory is dependent to a great extent on the support staff. It is essential that such personnel be given appropriate safety training.

Engineering and building maintenance services

Skilled engineers and craftsmen who maintain and repair the structure, facilities and equipment should have some knowledge of the type of the work in the laboratory and the laboratory safety regulations and procedures.

Equipment testing after certification or servicing, e.g. certifying biosafety cabinets after new filters have been installed, may be performed with supervision of a qualified biosafety officer. Copies of certification and service reports shall be retained by the safety office or administrative unit.

Laboratories or institutions that do not have internal engineering and maintenance services should establish good relationships with local service providers and familiarize them with the equipment and work in the laboratory.

Engineering and maintenance staff should only enter Biosafety Level 3 or Biosafety Level 4 laboratories with clearance and supervision by the biosafety officer and/or the laboratory supervisor.

Cleaning (domestic) services

Biosafety Level 3 and Biosafety Level 4 laboratories should be cleaned by the laboratory staff. Cleaning personnel should only enter Biosafety Level 3 or Biosafety Level 4 laboratories after appropriate training and with clearance and supervision by the biosafety officer and/or the laboratory supervisor.

20. Training programs

A continuous, on-the-job safety training program is essential to maintain safety awareness among laboratory and support staff. Laboratory supervisors, with the assistance of the biosafety officer and other resource persons, play a key role in staff training. The effectiveness of biosafety training, indeed all safety and health training, depends on management commitment, motivational factors, adequate initial job training, good communications and ultimately the organization’s goals and objectives. Annual refresher safety training for all staff may be appropriate at MOPH-designated facilities.

PART VIII

Safety checklist

21. Safety checklist

This checklist is intended to assist the assessment of microbiological laboratory safety and security status of biomedical laboratories.

Laboratory premises

1. Have guidelines for commissioning and certification been considered for facility construction or post-construction evaluations?
2. Do the premises meet national and local building requirements, including those relating to natural disaster precautions if necessary?
3. Are the premises generally uncluttered and free from obstructions?
4. Are the premises clean?
5. Are there any structural defects in floors?
6. Are floors and stairs uniform and slip-resistant?
7. Is the working space adequate for safe operation?
8. Are the circulation spaces and corridors adequate for the movement of people and large equipment?
9. Are the benches, furniture and fittings in good condition?
10. Are bench surfaces resistant to solvents and corrosive chemicals?
11. Is there a hand-washing sink in each laboratory?
12. Are the premises constructed and maintained to prevent entry and harborage of rodents and arthropods?
13. Are all exposed steam and hot water pipes insulated or guarded to protect personnel?
14. Is an independent power supply provided for essential equipment when there is a power outage?
15. Is access to laboratory areas restricted to authorized personnel?
16. Has a risk assessment been performed to ensure that appropriate equipment and facilities are available to support the work being considered?

Storage facilities

1. Are storage facilities, shelves, etc. arranged so that materials will not slide, collapse or fall?
2. Are storage facilities kept free from accumulations of rubbish, unwanted materials and objects that present hazards from tripping, fire, explosion and harborage of pests?
3. Are freezers and storage areas lockable?

Sanitation and staff facilities

1. Are the premises maintained in a clean, orderly and sanitary condition?
2. Is drinking-water available?
3. Are clean and adequate toilet (WC) and washing facilities provided separately for male and female staff?
4. Are hot and cold water, mild soap and towels provided?
5. Are separate changing rooms provided for male and female staff?
6. Is there accommodation (e.g. lockers) for street clothing by individual members of the staff?
7. Is there a staff room for lunch, etc.?
8. Are noise levels acceptable?
9. Are there appropriate containers for collection and disposal of general household trash?

Heating and ventilation

1. Is there a comfortable work temperature?
2. Are blinds fitted to windows that are exposed to full sunlight?
3. Is the ventilation adequate, e.g. at least six to eight changes of air per hour, in rooms with mechanical ventilation?
4. Are there HEPA filters in the ventilation system?
5. Do mechanical air supply vents compromise airflow in or around biosafety cabinets and chemical fume hoods?

Lighting

1. Is the general illumination adequate (e.g. 300–400 lx)?
2. Is task (local) lighting provided at work benches?
3. Are all areas well-lit, with no dark or poorly lit corners in rooms and corridors?
4. Are fluorescent or LED lights parallel to the benches?
5. Are fluorescent or LED lights color-balanced?

Services

1. Is each laboratory provided with enough sinks, water, electricity and gas outlets for safe working?
2. Is there an adequate inspection and maintenance program for fuses, circuit breakers, lights, cables, pipes, etc.?
3. Are faults corrected within a reasonable time?

4. Are internal engineering and maintenance services available with skilled engineers and craftsmen who also have some knowledge of the nature of the work in the laboratory?
5. Is the access of engineering and maintenance personnel to various laboratory areas controlled and documented?
6. If no internal engineering and maintenance services are available, have local engineers and builders been contacted and familiarized with the equipment and work of the laboratory?
7. Are cleaning services available?
8. Is the access of cleaning personnel to various laboratory areas controlled and documented?
9. Are information technology services available and secured?

Laboratory biosecurity

1. Has a qualitative risk assessment been performed to define risks that a security system should protect against?
2. Have acceptable risks and incident response planning parameters been defined?
3. Is the whole building securely locked when unoccupied?
4. Are doors and windows break-proof?
5. Are rooms containing hazardous materials and expensive equipment locked when unoccupied?
6. Is access to such rooms, equipment and materials appropriately controlled and documented?

Fire prevention and fire protection

1. Is there a fire alarm system?
2. Are the fire doors in good order?
3. Is the fire detection system in good working order and regularly tested?
4. Are fire alarm stations accessible?
5. Are all exits marked by proper, illuminated signs?
6. Is access to exits marked when the routes to them are not immediately visible?
7. Are all exits unobstructed by decorations, furniture and equipment and unlocked when the building is occupied?
8. Is access to exits arranged so that it is not necessary to pass through a high-hazard area to escape?
9. Do exitsall lead to an open space?
10. Are corridors, aisles and circulation areas clear and unobstructed to allow movement of staff and fire-fighting equipment?
11. Is all firefighting equipment and apparatus easily identified by an appropriate color code?

12. Are portable fire extinguishers maintained fully charged, in working order and kept in designated places at all times?
13. Are laboratory rooms with potential fire hazards equipped with appropriate extinguishers and/or fire blankets for emergency use?
14. If flammable liquids and gases are used in any room, is the mechanical ventilation sufficient to remove vapors before they reach a hazardous concentration?
15. Are personnel trained to respond to fire alarms and other emergencies?
16. Are there designated fire wardens?

Flammable liquid storage

1. Is the storage facility for bulk flammable liquids separated from the main building?
2. Is it clearly labelled as a fire-risk area?
3. Does it have a gravity or mechanical exhaust ventilation system that is independent of the main building system?
4. Are the light switches sealed or placed outside the building?
5. Are the interior light fixtures sealed to protect against ignition of vapors by sparking?
6. Are flammable liquids stored in proper, ventilated containers that are made ofnon-combustible materials?
7. Are the contents of all containers correctly described on the labels?
8. Are appropriate fire extinguishers and/or fire blankets placed outside but near tothe flammable liquid storage area?
9. Are “No Smoking” signs clearly displayed inside and outside the flammable liquidstorage area?
10. Are only minimum amounts of flammable substances stored in laboratory rooms?
11. Are they stored in properly constructed flammable storage cabinets?
12. Are these cabinets adequately labelled with “Flammable liquid – Fire hazard” signs?
13. Are personnel trained to properly use and transport flammable liquids?

Compressed and liquefied gases

1. Is each portable gas container legibly marked with its contents and correctly colorcoded?
2. Are compressed-gas cylinders and their high-pressure and reduction valves regularlyinspected?
3. Are reduction valves regularly maintained?
4. Is a pressure-relief device connected when a cylinder is in use?
5. Are protection caps in place when cylinders are not in use or are being transported?

6. Are all compressed gas cylinders secured so that they cannot fall, especially during natural disasters?
7. Are cylinders and liquid petroleum gas tanks kept away from sources of heat?
8. Are personnel trained to properly use and transport compressed and liquefied gases?

Electrical hazards

1. Are all new electrical installations and all replacements, modifications or repairsmade and maintained in accordance with the national electrical safety code?
2. Does the interior wiring have agrounded conductor (i.e. a three-wiresystem)?
3. Are labeled circuit-breakers and ground-fault interrupters fitted to all laboratory circuits?
4. Do all electrical appliances have independent electrical testing laboratory approval?
5. Are the flexible connecting cables of all equipment as short as practicable, in goodcondition and not frayed, damaged or spliced?
6. Is each electric outlet used for only one appliance (no adapters to be used)?

Personal protection

1. Is protective clothing of approved design and fabric provided for all staff for normal work, e.g. gowns, coveralls, aprons, gloves?
2. Is additional protective clothing provided for work with hazardous chemicals and radioactive and carcinogenic substances, e.g. rubber aprons and gloves for chemicals and for dealing with spillages; heat-resistant gloves for unloading autoclaves and ovens?
3. Are safety glasses, goggles and shields (visors) provided?
4. Are there eye-wash stations?
5. Are there emergency showers (drench facilities)?
6. Is radiation protection in accordance with national and international standards, including provision of dosimeters?
7. Are reusable respirators available, regularly cleaned, disinfected, inspected and stored in a clean and sanitary condition?
8. Are appropriate filters provided for the correct types of respirators, e.g. HEPA filters for microorganisms, appropriate filters for gases or particulates?
9. Are single-use respirators available?
10. Are all personnel that wear respirators in a medical surveillance program?
11. Are respirators fit-tested?

Health and safety of staff

1. Is there an occupational health service?
2. Are firstaid boxes provided at strategic locations?
3. Are qualified firstaid personnel available?
4. Are such firstaid personnel trained to deal with emergencies peculiar to the laboratory, e.g. contact with corrosive chemicals, accidental ingestion of poisons and infectious materials?
5. Are non-laboratory workers, e.g. domestic and clerical staff, given instruction about potential hazards in the laboratory and the material that is handled?
6. Are notices prominently posted giving clear information about the location of first-aid personnel, telephone numbers of emergency services, etc.?
7. Are women of childbearing age warned of the consequences of work with certain microorganisms, carcinogens, mutagens and teratogens?
8. Are women of childbearing age informed that if they areor suspect that they are pregnant, they should inform the appropriate member of the medical/scientific staff so that alternative working arrangements may be made for them, if necessary?
9. Is there an immunization program relevant to the work of the laboratory?
10. Are skin tests and/or radiological facilities available for staff who work with tuberculous materials or other materials requiring such measures?
11. Are proper illness and accident records maintained?
12. Are warning and accident prevention signs used to minimize work hazards?
13. Are personnel trained to follow appropriate biosafety practices?
14. Are laboratory staff encouraged to report potential exposures and injuries?

Laboratory equipment

1. Is all equipment certified safe for use?
2. Are procedures available for decontaminating equipment prior to maintenance?
3. Are biosafety cabinets and chemical fume hoodsannually or more frequently tested and serviced?
4. Are autoclaves and other pressure vessels regularly inspected?
5. Are centrifuge buckets and rotors regularly inspected?
6. Are HEPA filters changed when they are no longer functional?
7. Are pipettes used instead of syringes and needles whenever possible?
8. Is cracked and chipped glassware always discarded and not reused?
9. Are there safe receptacles for broken glass?
10. Are plastics used instead of glass when feasible?
11. Are sharps disposal containers available and being used?

Infectious materials

1. Are specimens received in a safe area?
2. Are records kept of incoming materials?
3. Are specimens unpacked in biosafety cabinets with attention to possible breakage and leakage?
4. Are gloves and other protective clothing worn when unpacking specimens?
5. Are personnel trained to ship infectious substances according to current national and/or international regulations?
6. Are work benches kept clean and tidy?
7. Are discarded infectious materials removed daily or more often and discarded safely?
8. Are all members of the staff aware of procedures for dealing with breakage and spillage of cultures and infectious materials?
9. Is the performance of sterilizers checked by the appropriate chemical, physical and biological indicators?
10. Is there a procedure for decontaminating centrifuges regularly?
11. Are safety buckets provided for centrifuges?
12. Is there special training for staff who work in high containment laboratories – Biosafety Level 3 and maximum containment laboratories – Biosafety Level 4?
13. Are appropriate disinfectants being used? Are they used correctly?

Chemicals and radioactive substances

1. Are incompatible chemicals effectively separated when stored or handled?
2. Are all chemicals correctly labelled with names and warnings?
3. Are chemical hazard warning charts prominently displayed?
4. Are spill kits provided?
5. Are staff trained to deal with spills?
6. Are flammable substances correctly and safely stored in minimal amounts in approved cabinets?

MOPH Laboratory Inspection Checklist Date:

A: Acceptable **U:** Unacceptable **N/A:** Not Applicable

Lab Information							
Name							
Executive Director							
Contact number							
Executive Director email							
Address							
Lab room numbers							
Lab Safety contact person							
Lab Safety contact telephone number							
Lab Safety contact email address							
Lab phone number							
Radiation	☐	**Biosafety level 2 or greater**	☐	**Lasers**	☐	**Animals**	☐

Chemical Types			
☐	Particularly Hazardous Substances (carcinogens, acute and reproductive toxins)	☐	Flammables
☐	Regulated Carcinogens	☐	Explosives
☐	Pyrophoric	☐	Peroxide Formers
☐	Water Reactive	☐	Corrosives

Personnel Information		
First Name	**Last Name**	**ID**

Emergency and Safety Information				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Emergency Assistance Information
☐	☐	☐		NFPA fire Diamond
☐	☐	☐		NFPA fire diamond updated with current occupants & emergency contacts

Fire Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Storage Clearance from ceiling: 18” with sprinklers, 24” without sprinklers
☐	☐	☐		Fire Extinguisher present/charged/accessible/tag updated; visible signage
☐	☐	☐		Power and gas supply emergency switches clearly identified and easily accessible

General Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		All emergency and evacuation notifications displayed
☐	☐	☐		Laboratory Emergency Procedures Protocol posted (emergency phone numbers, steps to take in case of emergency etc.)
☐	☐	☐		Exits/aisles/corridors are not blocked (24”minimum width)
☐	☐	☐		Laboratory doors kept closed
☐	☐	☐		Approved safety shower & eyewash station accessible within 10sec (travel distance <100feet)
☐	☐	☐		Emergency shower & eyewash station inspected monthly (visible signage)
☐	☐	☐		Clearance area around emergency shower at least 16” in each direction
☐	☐	☐		First aid kit present stocked & without expired products and the supply list inside
☐	☐	☐		Chemical spill material or kit available, trained staff
☐	☐	☐		Gas cylinder secured upright with double chains to a stable structure (i.e. wall or with clam shell/frame casing)
☐	☐	☐		Gas cylinder protection cap in place when not in use
☐	☐	☐		“No Food” sign posted on lab microwaves, “No Food”and “No Flammables” sign posted on lab Refrigerators; and “Food Only” on office microwaves and refrigerators
☐	☐	☐		Biohazard warnings on freezers, refrigerators, liquid nitrogen containers and storage units where biological materials are present
☐	☐	☐		All secondary solution containers filled in the laboratory have hazard labeling class information
☐	☐	☐		Sink available for hand washing
☐	☐	☐		Well controlled laboratory temperature
☐	☐	☐		Adequate lighting in the laboratory

Personal Protective Equipment				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Closed toe shoes, long pants and lab coats/gowns worn by laboratory personnel
☐	☐	☐		Protective gloves, matched to the hazard available
☐	☐	☐		Eye protection available & used
☐	☐	☐		Adequate supply of specialty protective equipment available (i.e. UV/IR glasses, face shields, lab aprons, cryogenic gloves)
☐	☐	☐		Hazardous waste disposal for contaminated personal protective equipment
☐	☐	☐		Lab coats only worn in the laboratory and are removed before entering offices, lunchrooms, restrooms and other non-laboratory general use areas

Housekeeping				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		No food or drink in lab areas
☐	☐	☐		Ceiling tiles in place and free of any water leaks or stains
☐	☐	☐		Garbage containers free of broken glass and hazardous materials
☐	☐	☐		Bench tops and storage areas uncluttered and orderly
☐	☐	☐		Secondary containment provided for floor storage of glass bottles that contain chemicals
☐	☐	☐		Minimal glassware on bench top
☐	☐	☐		Minimal glassware in sink
☐	☐	☐		Minimal glassware in chemical fume hood
☐	☐	☐		Proper waste disposal of sharps (broken glass, pipettes, needles, razors etc.)
☐	☐	☐		Sharps containers less than ¾ full
☐	☐	☐		Glassware free from cracks, chips and other defects
☐	☐	☐		Wiring on laboratory equipment in a good condition and secured along the wall and benches
☐	☐	☐		Interiors of refrigerators and freezers are free of chemical spills or contamination and with containers tightly closed
☐	☐	☐		Electrical cords and appliances away from flammables and water (sinks). No grouping plugs. Extension cords used only for computers.

Chemical Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Primary and secondary chemical containers labeled with identity and appropriate hazard warnings
☐	☐	☐		Materialdata sheets available for all chemicals present in the laboratory
☐	☐	☐		All chemicals well labeled, caped and stored in good condition
☐	☐	☐		Less than 2 gallons of flammable located outside flammable storage cabinets
☐	☐	☐		Maximum 60gallonsof flammable liquids per flammable storage cabinet, maximum 3 flammable storage cabinets/lab/fire area
☐	☐	☐		Flammable storage/refrigerators/freezers approved and labeled
☐	☐	☐		Minimal acids storage outside corrosive cabinet
☐	☐	☐		Strong acids and bases stored in secondary containers
☐	☐	☐		Incompatible materials properly segregated
☐	☐	☐		Chemicals stored safely according to seismic safety
☐	☐	☐		Combustible materials not stored with flammable chemicals
☐	☐	☐		Chemical storage cabinets clearly labeled (i.e. flammables, corrosives, etc.)
☐	☐	☐		Chemical containers in good condition
☐	☐	☐		Corrosive chemicals stored below eye level
☐	☐	☐		Ethers and other peroxide formers dated
☐	☐	☐		Water reactive chemicals segregated, contained and labeled
☐	☐	☐		Carcinogens segregated and stored in designated areas
☐	☐	☐		Pyrophoric chemicals segregated, contained and labeled
☐	☐	☐		Chemicals kept away from desks
☐	☐	☐		Hazardous materials used/stored in small quantities

Chemical Fume Hoods				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Certified within one year
☐	☐	☐		Proper type for current use
☐	☐	☐		Proper sash height indicated
☐	☐	☐		Sash at or below marked approval level
☐	☐	☐		Sash stoppers functional where present
☐	☐	☐		Hood illumination functional
☐	☐	☐		Audible/Visual alarm functional
☐	☐	☐		Minimal clutter in hood
☐	☐	☐		Functional fume hood: unblocked and uncluttered, no stock chemicals stored inside

Biohazard Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Valid Biosafety certification
☐	☐	☐		Personnel appropriately trained, including standard precautions
☐	☐	☐		Appropriate door signage for BSL-2 or greater
☐	☐	☐		Personnel immunized if required
☐	☐	☐		Biosafety Cabinets: certified within one year
☐	☐	☐		Biosafety Cabinets: proper disinfectant present for the type of work

Radiation Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Radioisotope permit posted
☐	☐	☐		Active benches, equipment, containers and storage area properly designated
☐	☐	☐		Radiation monitoring and detection equipment readily available and calibrated
☐	☐	☐		Personnel trained appropriately
☐	☐	☐		Radioactive materials securely stored according to procedures
☐	☐	☐		Dose rate at any occupied location outside the storage area or room does not exceed 2.5microSv/hr (250uR/hr)
☐	☐	☐		Personnel who handle more than 0.13mCi (open bench), 13mCi (Glove box) of radioiodine or 4KBq of tritium must go through biomonitoring process
☐	☐	☐		Fume hoods available for volatile radionuclide work, functional and certified within a year
☐	☐	☐		Personnel protective equipment and laboratory essentials are available, including gloves, absorbent pads, wipe test paper, radiation tape, decontamination solution etc.
☐	☐	☐		Contamination monitoring performed and recorded in log book (i.e. print out wipe test) seven days after working with unsealed nuclear substances
☐	☐	☐		Survey meter available for types of radiation work and it is functioning properly

Hazardous Waste Disposal				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Safety cans available and labeled for disposal of solvents
☐	☐	☐		Containers available and labeled for disposal of hazardous waste
☐	☐	☐		Waste manifest or tags attached to waste cans or containers
☐	☐	☐		Waste containers in good condition and kept closed
☐	☐	☐		Sturdy cart available for hazardous waste transport
☐	☐	☐		Hazardous waste in secondary containment
☐	☐	☐		Approved sharps disposal containers
☐	☐	☐		Radioactive waste properly disposed (i.e. liquid waste disposed into plastic container, liquid scintillation vials disposed separately, radiation symbols are removed/defaced from shipping packages, etc.)
☐	☐	☐		Hazardous biological wastes packaged, disinfected or sterilized
☐	☐	☐		Biohazard waste containers rigid, labeled and with lids
☐	☐	☐		Waste disposed when full or within 90 days, whichever is sooner
☐	☐	☐		Dry hazardous waste double-bagged in transparent bags
☐	☐	☐		Hazardous/chemical materials not found in regular trash

Seismic Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Shelving and file cabinets 5’ or over anchored/bolted
☐	☐	☐		Storage shelves have seismic restrains (i.e. lips, bars, bungee cords)
☐	☐	☐		High overhead storage is secured
☐	☐	☐		Heavy items stored on lower shelves
☐	☐	☐		Aisles and exits free from obstruction

Mechanical & Electrical Safety				
A	**U**	**N/A**	**Comments**	
☐	☐	☐		Electrical panels unobstructed by 3 ft (0.9 m)
☐	☐	☐		Permanent equipment (placed more than 6months is considered permanent) has permanent wiring (no extension cords)
☐	☐	☐		Adequate extension cords on temporary equipment
☐	☐	☐		Electrical outlets within 6ft (1.8 m) of a sink or wet area equipped with ground-fault circuit interrupters
☐	☐	☐		Plugs, cords, outlets in good condition
☐	☐	☐		All equipment grounded via 3-prog plugs or polarized 2-prong plugs
☐	☐	☐		High voltage equipment (>600V) labeled, grounded and insulated
☐	☐	☐		Electric panels accessible
☐	☐	☐		Nothing posted on electrical panels

Comments:

PART IX

References and Annexs

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ANNEX 1 - First aid

First aid is the skilled application of accepted principles of medical treatment at the time and place of an accident. It is the approved method of treating a casualty until he or she is placed in the care of a doctor for definitive treatment of the injury. The minimum firstaid equipment consists of a firstaid box, protective clothing and safety equipment for the person rendering the first aid and eye irrigation equipment.

The firstaid box - hospital

The firstaid box should be constructed from materials that will keep the contents dust- and damp-free. It should be kept in a prominent position and be easily recognized.

The firstaid box should contain:

1. Instruction sheet giving general guidance.
2. Individuallywrapped sterile adhesive dressings in a variety of sizes.
3. Sterile eyepads with attachment bandages.
4. Triangular bandages.
5. Sterile wound coverings.
6. Safety pins.
7. A selection of sterile but un-medicated wound dressings.
8. An authoritative first-aid manual, e.g. one issued by the International Red Cross.

Protective equipment for the person rendering first aid includes:

1. Mouthpiece for mouth-to-mouth resuscitation.
2. Gloves and other barrier protections against blood exposure. (4)
3. Clean-up kit for blood spills (see Chapter 13 of the manual).

Eye irrigation equipment should also be readily available and staff trained in its correct use.

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The firstaid box - workplace

The following list sets forth the minimally acceptable number and type of firstaid supplies for firstaid kits required by the United States Occupational Safety and Health Administration (OSHA) 29 CFR 1910.266. The contents of the firstaid kit should be adequate for small work sites, consisting of approximately two to three employees. When larger operations or multiple operations are being conducted at the same location, additional firstaid kits should be provided at the work site or additional quantities of supplies should be included in the firstaid kits:

1. Gauze pads (at least 4 x 4 inches).
2. Two large gauze pads (at least 8 x 10 inches).
3. Box adhesive bandages (band-aids).
4. One package gauze roller bandage at least 2 inches wide.
5. Two triangular bandages.
6. Wound cleaning agent such as sealed moistened towelettes.
7. Scissors.
8. At least one blanket.
9. Tweezers.
10. Adhesive tape.
11. Nitrile or latex gloves.
12. Resuscitation equipment such as resuscitation bag, airway, or pocket mask.
13. Two elastic wraps.
14. Splint.
15. Directions for requesting emergency assistance.

ANNEX 2 - Immunization of staff

The risks of working with particular agents should be fully discussed with individual researchers. The local availability, licensing state and utility of possible vaccines and/ or therapeutic drugs (e.g. antibiotic treatments) in case of exposure should be evaluated before work with such agents is started. Some workers may have acquired immunity from prior vaccination or infection.

If a particular vaccine or toxoid is locally licensed and available, it should be offered after a risk assessment of possible exposure and a clinical health assessment of the individual has been completed.

Facilities for specific clinical case management following accidental infections should also be known by the laboratory staff.

ANNEX 3 - Equipment safety

Certain items of equipment may create microbiological hazards when they are used. Other items are specifically designed to prevent or reduce biological hazards (see Chapter 11 of the manual).

Equipment that may create a hazard

Table A3-1 lists equipment and operations that may create hazards and suggests how such hazards may be eliminated or reduced.

Table A3-1. Equipment and operations that may create hazards

EQUIPMENT	HAZARD	HOW TO ELIMINATE OR REDUCE THE HAZARD
Hypodermic Needles	Accidental inoculation aerosol or spillage	• Do not recap or clip needles. • Use a needle-locking type of syringe to prevent separation of needle and syringe, or use a disposable type where the needle is an integral part of the syringe unit. • Use standard microbiological practices, e.g.: – Fill the syringe carefully to minimize air bubbles and frothing of inoculum. – Avoid using syringes to mix infectious liquids; if used, ensure that the tip of the needle is held under the surface of the fluid in the vessel and avoid excessive force. – wrap the needle and stopper in a cotton gauze pledget moistened with an appropriate disinfectant before withdrawing the needle from a rubber-stoppered bottle. – Expel excess liquid and air bubbles from the syringe vertically into a cotton gauze pledget moistened with an appropriate disinfectant or into a small bottle

		containing cotton. • Use a biosafety cabinet for all operations with infectious material.
		• Restrain animals while they are being inoculated. Use blunt needles or cannulas for intranasal or oral inoculation. Use a biosafety cabinet. • Autoclave after use and ensure proper disposal. If a disposable needle and syringe unit is used, do not disassemble prior to autoclaving.
Centrifuges	Aerosols, splashing and tube breakage	• Use sealable buckets (safety cups) or sealed rotors. Open buckets or rotors after aerosols have settled (30 min) or in a biosafety cabinet.
Ultracentrifuges	Aerosols, splashing and tube breakage	• Install a HEPA filter between centrifuge and vacuum pump. • Maintain a logbook of operating hours for each rotor and a preventive maintenance program to reduce risk of mechanical failure. • Load and unload buckets or rotors in a biosafety cabinet.
Anaerobic jars	Explosion, dispersing infectious materials	• Ensure integrity of wire capsule around catalyst.
Desiccators	Implosion, dispersing glass fragments and infectious materials	• Place in a stout wire cage.
Homogenizer, tissue grinders	Aerosols, leakage and container breakage	• Operate and open equipment in a biosafety cabinet when possible. • Use specially designed models that prevent leakage from rotor bearings and O-ring gaskets, or use a stomacher. • Before opening the blender bowl, wait 30 min to allow the aerosol cloud to settle. Refrigerate to condense aerosols. • If manual tissue grinders are used, hold tube in a wad of absorbent material.
Sonicators, Ultrasonic cleaners	Aerosols, impaired hearing, dermatitis	• Operate and open equipment in a biosafety cabinet or sealed unit when possible. • Ensure sound deadening insulation protects

		against subharmonics. • Wear gloves to protect skin against chemical effects of detergents.
Culture mixers shakers, agitators	Aerosols, splashing and spillage	• Operate in a biosafety cabinet or specially designed primary containment. • Use heavy-duty screw-capped culture flasks, fitted with filter-protected outlets, if necessary, and make sure they are well secured.
Freeze-dryers (lyophilizes)	Aerosols and direct contact contamination	• Use O-ring connectors to seal the unit throughout. • Use HEPA air filters to protect vacuum lines. • Use a satisfactory method of decontamination, e.g. chemical. • Provide an all-metal moisture trap and a vapor condenser. • Carefully inspect all glass vacuum vessels for surface scratches. Use only glassware designed for vacuum work.
Water baths.	Growth of microorganisms. Sodium azide forms explosive compounds with some metals	• Ensure regular cleaning and disinfection • Do not use sodium azide for preventing growth of organisms. • Consider use of an iodophore disinfectant.

In addition to microbiological hazards, safety hazards associated with equipment should also be anticipated and prevented. Table A3-2 lists examples of some of the causes of accidents

Table A3-2. Common causes of equipment-related accidents

ACCIDENT	ACCIDENT CAUSE	REDUCING OR ELIMINATING THE HAZARD
Faulty design or construction		
Electrical fires in incubators	No over-temperature cut-out	• Compliance with national standards
Electrical shock	Failure to provide reliable grounding	
Improper use		
Centrifuge accident	Failure to balance buckets on swinging bucket rotors	• Train and supervise staff
Anaerobic incubator explosion	Use of incorrect gas	• Train and supervise staff
Improper adaptation		
Explosion in domestic vacuum flask	Improper transport of liquid nitrogen	• Use of specially designed equipment.
Explosion in domestic-type refrigerator	Dangerous chemical not stored in spark-/explosion- proof container, e.g. diethyl ether with leaking screw cap	• Store low-flashpoint solvents and extracts only in spark-/ explosion-proof refrigerators or cabinets.
Lack of proper maintenance		
Fire in flame photometer	Incorrect reassembly of components during maintenance	• Train and supervise staff.

ANNEX4 - Humanand Animal etiological agents

This annex includes those biological agents known to infect or injure humans as well as selected animal agents that may pose potential risks. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*5thEdition, HHS Publication No. (CDC) 21-1112, Revised December 2009.

Table A4-1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

United States

The US Centers for Disease Control and Prevention (CDC)/National Institutes of Health (NIH) biosafety entitled “Biosafety in Microbiological and Biomedical Laboratories (BMBL)”no longer classifies agents by risk group. Instead, Section VIII “Agent Summary Statements”, lists agents by type (bacterial, fungal, parasitic, rickettsial, viral, arboviruses and related zoonotic viruses, toxins and prions) in alphabetical order,
http://www.cdc.gov/biosafety/publications/bmbl5/

1. Section VIII – A: Bacterial Agents.
2. Section VIII – B: Fungal Agents.
3. Section VIII – C: Parasitic Agents.
4. Section VIII – D: Rickettsial Agents.
5. Section VIII – E: Viral Agents.

6. Section VIII – F: Arboviruses and Related Zoonotic Viruses.
7. Section VIII – G: Toxin Agents.
8. Section VIII – H: Prion Diseases.

Select agents

The United States of America, Health and Human Services (HHS) and United States Department of Agriculture (USDA) in 7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73 list the following biological agents and toxins determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. Excluded agents and toxins are available online at: http://www.selectagents.gov/SelectAgentsandToxinsExclusions.html

Table A4-2. Select Agents

HHS SELECT AGENTS AND TOXINS	**OVERLAP SELECT AGENTS AND TOXINS**
Abrin	Bacillus anthracis*
Botulinum neurotoxins*	Bacillus anthracis Pasteur strain
Botulinum neurotoxin producing species of *Clostridium**	Brucella abortus
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence$X_1CCX_2PACGX_3X_4X_5X_6CX_7$)[1]	Brucella melitensis
Coxiella burnetii	Brucella suis
Crimean-Congo haemorrhagic fever virus	Burkholderia mallei*
Diacetoxyscirpenol	Burkholderia pseudomallei*
Eastern Equine Encephalitis virus[3]	Hendra virus
Ebola virus*	Nipah virus
*Francisella tularensis**	Rift Valley fever virus
Lassa fever virus	Venezuelan equine encephalitis virus[3]
Lujo virus	

Marburg virus*
Monkeypox virus[3]

Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)

Ricin

Rickettsia prowazekii

SARS-associated coronavirus (SARS-CoV)

Saxitoxin

South American Haemorrhagic Fever viruses:

- Chapare
- Guanarito
- Junin
- Machupo
- Sabia

Staphylococcal enterotoxins A,B,C,D,E subtypes

T-2 toxin

Tetrodotoxin

Tick-borne encephalitis complex (flavi) viruses:

- Far Eastern subtype
- Siberian subtype

Kyasanur Forest disease virus

Omsk hemorrhagic fever virus

Variola major virus (Smallpox virus)*

Variola minor virus (Alastrim)*

USDA SELECT AGENTS AND TOXINS

African horse sickness virus

African swine fever virus

Avian influenza virus[3]

Classical swine fever virus

Foot-and-mouth disease virus*

Goat pox virus

Lumpy skin disease virus

Mycoplasma capricolum[3]

Mycoplasma mycoides[3]

Newcastle disease virus[2,3]

Peste des petits ruminants virus

Rinderpest virus*

Sheep pox virus

Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ)SELECT AGENTS AND TOXINS

Peronosclerospora philippinensis (Peronosclerospora sacchari)

Phoma glycinicola (formerly Pyrenochaeta glycines)

Ralstonia solanacearum

*Yersinia pestis**	Rathayibacter toxicus Sclerophthora rayssiae Synchytrium endobioticum Xanthomonas oryzae

*Denotes Tier 1 Agent

[1] C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins α-MI and α-GI (shown above) as well as α-GIA, Ac1.1a, α-CnIA, α-CnIB; X1 = any amino acid(s) or Des-X; X2 = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X3 = Arginine or Lysine; X4 = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X5 = Tyrosine, Phenylalanine, or Tryptophan; X6 = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X7 = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example, if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

[2] A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (Gallus gallus) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

[3] Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus , west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies Mycoplasma capricolum except subspecies capripneumoniae (contagious caprine pleuropneumonia), all subspecies Mycoplasma mycoides except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia), and any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, provided that the individual or entity can verify that the agent is within the exclusion category.

Canada

Public Health Agency of CanadaPathogen Safety Data Sheets (PSDSs) (previously titled Material Safety Data Sheets for infectious substances) are technical documents that describe the hazardous properties of a human pathogen and recommendations for work involving these agents in a laboratory setting. These documents been produced as educational and informational resources for laboratory personnel working with these infectious substances. The

list of safety lists by pathogen name is available online at:
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

ANNEX 5 - Chemicals: hazards and precautions

This annex lists the basic health and safety information, data and appropriate safety precautions for a selected number of chemicals found commonly in health-care and research laboratories. The list is not exhaustive and the absence of any particular chemical does not imply that it is non-hazardous. All laboratory chemicals should be treated with caution and in ways that will minimize exposure.

Table A5-1. Chemicals: hazards and precautions

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Acetaldehyde CH_3CHO	Colourless liquid or gas with a pungent, fruity odour; m.p. –121 °C b.p. 21 °C.	Mild eye and respiratory tract irritation. Effects on the central nervous system, respiratory tract and kidneys. Possible carcinogen.	Extremely flammable; vapour/air mixtures are explosive; flash point –39 °C flammable range 4–57%.	No open flames, no sparks, no smoking, no contact with hot surfaces. Store in tightly sealed containers in areas separate from oxidizers; store only if stabilized. Use in exhaust cupboard or with good ventilation. Wear rubber gloves, safety goggles, and respiratory protection.	Can form explosive peroxides in contact with air. May polymerize under influence of acids, alkaline materials, in the presence of trace metals. A strong reducing agent, reacts violently with oxidants, with various organic substances, halogens, sulfuric acid and amines.	
Acetic acid CH_3CO_2H	Colourless liquid with pungent odour; m.p. 17 °C b.p. 118 °C; miscible with water.	Corrosive; causes severe burns; irritating vapour. Effects may be delayed.	Flammable; flashpoint 40 °C flammable range 5.4–16%.	Do not breathe fumes. In case of contact with eyes rinse immediately with water and seek medical advice. Wear nitrile gloves and eye protection.	Violent or explosive reaction with oxidizers.	
Acetic anhydride $(CH_3CO)_2O$	Colourless liquid with a strong pungent, vinegar-like odour; m.p. –73 °C b.p. 139 °C.	Severe irritation of eyes and upper respiratory tract irritation; corrosive action. Effects may be delayed.	Flammable; evolves irritation or toxic fumes or gases in a fire; flashpoint 49 °C explosive limits 2.7–10.3%.	No open flames, no sparks, no smoking. Prevent skin and eye contact.	Reacts violently with boiling water, steam, strong oxidants, alcohols, amines, strong bases and many other compounds. Attacks many metals in presence of water.	

Acetone CH_3COCH_3	Colourless volatile liquid with sweetish odour; m.p. –95 °C, b.p. 56 °C.; miscible with water.	Slight eye, nose and throat irritation. Inhalation may cause dizziness, narcosis and coma.	Highly flammable; flashpoint –18 °C explosive limits 2.2–12.8%.	Keep container in well-ventilated area; keep away from sources of ignition. Do not breathe vapour. Use respiratory protection; wear eye protection.	Reacts violently with oxidizers (e.g. chromic and nitric acids) and chloroform in the presence of base. Incompatible with concentrated sulfuric and nitric acid mixtures.	Earth/ground large containers and vessels to prevent static electricity.
Acetonitrile CH_3CN	Colourless liquid with an aromatic odour; m.p –46 °C b.p. 82 °C.	Respiratory, eye and skin irritation. Exposure may result in convulsions unconsciousness, cyanide poisoning.	Highly flammable; flashpoint 12.8 °C explosive limits 3.0–16%.	No open flames, no sparks, no smoking, no contact with oxidants. Use only in areas free of ignition sources. Store in tightly sealed containers in areas separate from oxidizers. Work with exhaust ventilation. Avoid skin, eye and mucous membrane contact. Use respiratory protection and rubber gloves.	Reacts with aqueous acids and bases, producing toxic fumes. Reacts with strong oxidants. Attacks some forms of plastic, rubber and coatings. Decomposes on burning producing hydrogen cyanide and nitrogen oxides.	
Acetylene $HC{\equiv}CH$	Colourless gas with a faint, ethereal or garlic-like odour; shipped under pressure, dissolved in acetone; m.p. –81 °C sublimes at –84 °C.	Simple asphyxiant; frostbite on skin contact.	Extremely flammable; flammable range 2.5–100%.	For skin protection use cold-insulating gloves and safety goggles or face shield. No open flames, no sparks, no smoking. Work with local exhaust ventilation, explosion-proof electrical equipment and lighting.	Strong reducing agent; reacts violently with oxidants and with fluorine or chlorine under influence of light. Reacts with copper, silver and mercury or their salts, forming shock-sensitive compounds.	

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Acrolein CH_2=CHCHO	Colourless or yellow liquid with a piercing, disagreeable odour; m.p. –87 °C b.p. 53 °C.	Lacrimation. Severe respiratory irritation; lung oedema at high exposure levels. Effects may be delayed.	Highly flammable; flashpoint –26 °C explosive limits 2.8–31%.	Prevent skin and eye contact. Work in fume cupboard or with good ventilation.	Oxidizers, acids, alkalis, ammonia, amines. Polymerizes readily unless inhibited, usually with hydroquinone. May form shock-sensitive peroxides over time.	
Ammonia solutions	Colourless liquid with pungent odour; for gas: m.p. –78 °C b.p. –33 °C; for 25% solution: m.p. –58 °C b.p. 38 °C; miscible with water.	Corrosive to eyes, respiratory system and skin on ingestion; lung oedema at high levels of exposure to gas or vapour.	As ammonia gas; flammable range 15–28%.	Keep container tightly closed. In case of contact with eyes, rinse immediately and seek medical advice. Work in fume cupboard. Wear rubber or plastic gloves and chemical-grade goggles.	Reacts violently with heavy metals such as mercury and their salts to form explosive products.	
Aniline $C_6H_5NH_2$	Colourless to brown, oily liquid with an aromatic amine-like odour; m.p. –6 °C b.p. 185 °C.	Cyanosis due to methaemoglobinaemia. Eye and skin irritation. May be absorbed through the skin; repeated or prolonged exposure may cause sensitization.	Combustible; flashpoint 70 °C explosive range 1.2–11%.	Store in tightly sealed containers in areas separate from oxidizers. Prevent skin and eye contact. Work with local exhaust ventilation or respiratory protection, protective gloves, protective clothing, face shield.	Strong oxidizers, strong acids.	
Auramine 4,4'-Carbonoimidoylbis (N,N-dimethylbenzenamine)	Yellow flakes or powder; m.p. 136 °C; insoluble in water.	Harmful by ingestion, inhalation and skin contact. May cause eye or skin irritation. Possible carcinogen.		Avoid skin contact and inhalation of dust. Wear rubber or plastic gloves and chemical-grade goggles. Work in fume cupboard or wear dust respirator.	Strong oxidizing agents.	

Benzene C_6H_6	Colourless volatile liquid with characteristic aromatic odour; m.p. 6 °C b.p. 80 °C.	Inhalation of vapour causes effects on central nervous system resulting in vertigo and headache; at high concentrations, unconsciousness and death. Risk of aplastic anaemia, leukaemia, liver damage on prolonged or chronic exposure. May be absorbed through skin.	Highly flammable; flashpoint –11 °C flammable range 1.3–8%.	Keep container in well-ventilated area and *away* from sources of ignition. Work in fume cupboard or hood with adequate ventilation. Wear eye protection and nitrile or PVC gloves. Prevent formation of electrical charges by earthing/ grounding.	Can react violently with oxidizers including chromic acid, potassium permanganate and liquid oxygen.	
Benzidine 1,1'-Biphenyl-4,4'-diamine	Light yellow powder; m.p. 128 °C b.p. 400 °C slightly soluble in water but very soluble in acids and organic solvents.	May be absorbed through skin. May cause bladder cancer. Avoid all exposure.	Combustible, gives off toxic fumes (gases) in a fire.	Avoid all exposure. Wear eye and skin protection. Work in fume cupboard with exhaust ventilation.	Use is prohibited or legally controlled in many countries.	
Bromine Br_2	Dark reddish-brown fuming liquid with pungent odour; m.p. –7.2 °C b.p. 58.8 °C.	Corrosive. Vapour is corrosive to eyes and respiratory tract; inhalation may cause lung oedema and effects on central nervous system. Eye contact can cause blurred vision, redness, pain, severe tissue burns.	Not combustible but enhances combustion of other substances. Many reactions may cause fire or explosion. Heating will cause rise in pressure with risk of burning.	Use in closed system and with ventilation. Wear protective gloves and clothing, safety goggles, face shield or eye protection in combination with respiratory protection.	Strong oxidant, reacts violently with combustible and reducing materials. Reacts violently with aqueous ammonia, oxidants, metals, organic compounds and phosphorus.	Attacks some forms of plastic, rubber and coatings.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Carbon dioxide (solid; "dry ice") CO_2	Translucent white solid at –79 °C; sublimes to gas at ambient temperature.	Risk of asphyxiation in confined or poorly ventilated areas; contact with solid "dry ice" causes frostbite.		Wear protective insulated gloves. Store only in ventilated room or area in open container.	Alkali metals, strong bases.	
Carbon tetra-chloride CCl_4	Colourless liquid with characteristic ether-like odour; m.p. –23 °C b.p. 76.5 °C.	May be absorbed through skin; may cause dermatitis on prolonged exposure. Eye irritation. May cause liver and kidney damage and central nervous system disturbances resulting in headache, nausea, slight jaundice, loss of appetite and narcosis. An animal carcinogen.	Not combustible. Gives off irritating or toxic fumes or gases in a fire.	Avoid all contact. Work with ventilation, local exhaust or respiratory protection; use nitrile gloves and protective clothing, face shield or eye protection in combination with respiratory protection.	On contact with hot surfaces or flames, decomposes forming toxic and corrosive fumes and gases (hydrogen chloride, chlorine, phosgene). Reacts with some metals such as aluminium, magnesium, zinc.	
Chlorine Cl_2	Greenish-yellow gas with pungent odour; m.p. –101 °C b.p. –34 °C.	Corrosive to *eyes*, skin and respiratory tract. Inhalation of gas may cause pneumonitis and lung oedema, resulting in reactive airways dys-function syndrome (RADS). Rapid evaporation of the liquid may cause frostbite. High exposures may result in death. Effects may be delayed; medical observation indicated.	Not combustible but enhances combustion of other substances.	Work with closed system and ventilation. Wear cold-insulating gloves, protective clothing, safety goggles or eye protection in combination with respiratory protection.	Solution in water is a strong acid, reacts violently with bases and many organic compounds, acetylene, butadiene, benzene and other petroleum fractions, ammonia, hydrogen, sodium carbide, turpentine and finely divided metals causing fire and explosion hazard.	Attacks many metals in presence of water. Attacks plastics, rubber and coatings.

Chlorine dioxide ClO_2	Yellow to red gas or a red-brown liquid; m.p –59 °C b.p. 10 °C.	Severe irritation of eyes, skin and respiratory tract; inhalation of gas may cause lung oedema. Effects may be delayed: medical observation indicated.	Not combustible but enhances combustion of other substances: may explode on heating, on exposure to sunlight or if subjected to shock and sparks.	Work in closed system with ventilation. Wear protective gloves and clothing, safety goggles or eye protection in combination with respiratory protection.	A strong oxidant; reacts violently with combustible and reducing materials. Reacts violently with phosphorus, potassium hydroxide, sulfur, ammonia, methane, phosphine and hydrogen sulfide.	
Chloroform $CHCl_3$	Colourless volatile liquid with characteristic odour; m.p. –63 °C b.p. 61 °C; slightly soluble in water.	Harmful by inhalation, ingestion and skin contact; skin irritation. May cause effects on liver, kidneys and central nervous system resulting in headache, nausea, slight jaundice, loss of appetite, narcosis. Prolonged or chronic exposure causes cancer in animals; suspected human carcinogen.		Wear protective clothing, nitrile gloves and eye protection. Work in a fume cupboard.	Strong bases; some metals such as aluminium or magnesium, zinc powder; strong oxidizers.	When heated to decomposition, forms phosgene gas. Attacks plastics, rubber.
Chromic acid CrO_3 Chromium VI oxide	Dark red odourless flakes or powder often used in aqueous solutions; m.p. 197 °C.	Irritation of eyes, skin and respiratory system. Repeated or prolonged contact with skin may cause dermatitis, chrome ulcers and skin sensitization. Inhalation may cause asthma-like reactions. May cause perforation of nasal septum. Human carcinogen.	Decomposes above 250 °C to chromic oxide and oxygen with increased fire hazard. Many reactions may cause hazards.	Prevent skin and eye contact; avoid inhalation of fine dust and mist. Work with ventilation, local exhaust or respiratory protection.	In aqueous solution is a strong acid which reacts with bases and is corrosive. Strong oxidant, reacts with combustible, organic or other readily oxidizable materials (paper, wood, sulfur, aluminium, plastics etc.). Corrosive to metals.	

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Copper Cu	Reddish, lustrous, malleable, odourless solid; red powder, turns green on exposure to moist air; m.p. 1083 °C b.p. 2567 °C.	Inhalation of copper fume may cause metal fume fever.	Combustible.	Work with local exhaust or repiratory protection, protective gloves and goggles.	Shock-sensitive compounds are formed with acetylenic compounds, ethylene oxides azides and hydrogen peroxide. Reacts with strong oxidants like chlorates, bromates and iodates, causing explosion hazard.	
Cyanogen bromide BrCN	Colourless or white crystals, with pungent odour; m.p. 52 °C b.p. 61 °C.	Severe eye, skin and respiratory tract effects; inhalation of vapour may cause lung oedema which may result in convulsions, unconsciousness, respiratory failure and death.	Not combustible but forms flammable gas on heating. Gives off irritating or toxic fumes or gases in a fire.	Work in closed system with ventilation. Wear protective gloves and protective clothing, safety goggles face shield or eye protection in combination with respiratory protection.	Decomposes on heating and on contact with acids producing highly toxic and flammable hydrogen cyanide and corrosive hydrogen bromide. Reacts with strong oxidants. Reacts slowly with water and moisture to produce hydrogen bromide and hydrogen cyanide. Attacks many metals in the presence of water.	
Cytochalasin (A–J)	White powder; m.p. varies.	Toxic by ingestion, inhalation or absorption through skin. May cause congenital fetal malformation.		Avoid contact with eyes, skin, clothing; wear chemical-grade goggles and rubber or plastic gloves.	Strong oxidizing agents.	

Diethyl ether $C_2H_5OC_2H_5$	Colourless highly volatile liquid with sweet characteristic odour; m.p. –116 °C b.p. 34 °C; slightly soluble in water.	Irritation of *eyes* and respiratory tract. May affect central nervous system causing drowsiness and unconsciousness. Repeated inhalation may cause addiction.	Extremely flammable; flashpoint –45 °C flammable range 1.7–48%.	Keep container in well-ventilated area; keep away from sources of ignition; earth/ground containers to prevent static electrical discharges. Work in fume cupboard. Wear nitrile gloves to prevent defatting of skin.	Exposure to air and light may result in formation of explosive peroxides. Can react violently with oxidizers and halogens.
Dimethylamine $(CH_3)_2NH$	Colourless volatile liquefied gas with pungent odour; m.p. –93 °C b.p. 7 °C; miscible with water.	Severe irritation of *eyes* and respiratory system; inhalation may cause lung oedema. Rapid evaporation may cause frostbite. Solution is corrosive to eyes and skin.	Extremely flammable; flashpoint –26 °C flammable limits 2.8–14%. Solution highly flammable; flashpoint –18 °C.	Keep away from sources of ignition; in case of contact with eyes rinse immediately and seek medical advice. Work in fume cupboard. Wear nitrile gloves and chemical-grade goggles.	Can react with oxidizers, mercury.
2,4-Dinitro-phenyl-hydrazine $C_6H_3(NO_2)_2$-$NHNH_2$ 1-Hydrazino-2,4-dinitro-benzene	Orange-red crystalline powder; m.p. 200 °C; slightly soluble in water.	Irritation of skin and *eyes*. Harmful by ingestion, inhalation and skin contact.		Keep moist to reduce explosion risk. Wear dust respirator, rubber or plastic gloves and chemical-grade goggles.	Can react vigorously with oxidizers and reducers.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Dioxane $C_4H_8O_2$ Diethylene dioxide	Colourless liquid, with characteristic odour; m.p. 12 °C b.p. 101 °C.	Irritation of eyes and respiratory tract. May affect central nervous system resulting in headache, nausea, cough, sore throat, abdominal pain, dizziness, drowsiness, vomiting, unconsciousness. May be absorbed through skin. Kidney and liver damage. Probably a human carcinogen.	Highly flammable; distant ignition possible; as a result of flow, agitation, etc., electrostatic charges can be generated.	Work with ventilation, local exhaust. No open flames, no sparks, no smoking, no contact with strong oxidants or hot surfaces. Do not use compressed air for filling, discharging or handling; use non-sparking tools. Wear protective gloves, clothing, face shield or eye protection, in combination with respiratory protection.	Can form explosive peroxides. Reacts vigorously with strong oxidants and concentrated strong acids. Reacts explosively with some catalysts. Attacks many plastics.	
Ethanol CH_3CH_2OH	Colourless volatile liquid with slight, characteristic odour; m.p. –117 °C b.p. 79 °C; miscible with water.	Harmful if ingested. Irritation of eyes. May affect central nervous system.	Highly flammable; flashpoint 12 °C flammable limits 3–19%.	Keep container tightly closed; keep away from ignition sources.	Reacts violently with strong oxidizers.	
Ethanolamine $H_2NCH_2CH_2OH$ 2-Amino-ethanol	Colourless non-volatile viscous liquid with ammoniacal odour; m.p 10 °C b.p. 171 °C; miscible with water.	Corrosive to eyes, respiratory system and skin. May cause skin sensitization.	Flashpoint 85 °C.	Wear rubber or plastic gloves and eye protection.	Reacts with strong oxidizers.	

Formaldehyde solution (37–41% formaldehyde with 11–14% methanol) HCHO	Colourless liquid with a pungent odour; b.p. 96 °C; miscible with water.	Severely irritation of eyes and skin, irritation of respiratory tract; prolonged exposure to the vapour may cause asthma-like symptoms, conjunctivitis, laryngitis, bronchitis or broncho-pneumonia. May cause sensitization by skin contact. Possible risk of irreversible health effects. Possible carcinogen.	Flashpoint 50 °C.	Wear protective clothing such as plastic apron, rubber or plastic gloves and chemical-grade goggles. Work in fume cupboard or well-ventilated area.	Can react vigorously with oxidizers, with nitromethane to produce explosive products, with hydrochloric acid to produce the potent carcinogen *bis* (chloromethyl) ether.	Concentrated formaldehyde solutions become cloudy if stored below 21 °C and should be kept at 21–25 °C. Dilute solutions (1–5%) and medium-strength solutions (5–25%) retain many of the hazards of the concentrated form.
Glutaraldehyde $OHC(CH_2)_3CHO$	Colourless or pale yellow solution with pungent odour; m.p. –14 °C b.p. 189 °C; miscible with water.	Severe irritation of eyes and upper respiratory tract; prolonged inhalation exposure or skin contact may cause sensitization.		Work in fume cupboard or well-ventilated area. Wear rubber or plastic gloves and eye protection.	Can react vigorously with oxidizers.	Often supplied in aqueous solution at various concentrations with added stabilizer to enhance stability.
Hydrochloric acid (10–37%) HCl Hydrogen chloride	Colourless fuming liquid with a pungent odour; b.p. –121 °C; miscible with water.	Corrosive to eyes, respiratory system and skin; repeated inhalation of vapour can cause chronic bronchitis.		Do not breathe fumes; use respiratory protection. In case of contact with eyes, rinse immediately with water and seek medical advice; in case of contact with skin, wash immediately with plenty of water. Work in fume cupboard. Wear rubber or plastic gloves and eye protection (spectacles or goggles).	Reacts violently with bases (solids and concentrated solutions), explosively with solid potassium permanganate. Gives off toxic or explosive gases on contact with many metals.	Releases highly toxic fumes in fires.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Hydrogen peroxide H_2O_2	Colourless liquid; m.p. –39 °C (70%) b.p. 125 °C (70%); miscible with water, supplied in aqueous solution at various concentrations.	Corrosive at high concentration (60%), and at low concentration (6%) if contact with skin is prolonged. Dilute solutions are irritating to eyes, respiratory system and skin.	Oxidizing agent; contact with combustible material can cause fire.	In case of contact with skin, wash immediately with plenty of water. Wear nitrile gloves and eye protection if concentration exceeds 20%.	Reacts vigorously with a variety of chemical reagents including oxidizers and bases. Attacks most metals or their salts, flammable liquids and other combustible materials (paper, textiles), aniline and nitromethane.	Can decompose evolving oxygen, causing pressure rise in container. Store in dark, cool place. Do not use metallic containers or equipment, e.g. brass, copper, iron.
Hydrogen sulfide H_2S	Colourless gas with a strong odour of rotten eggs; b.p. –60 °C m.p. –85 °C.	May cause effects on central nervous system resulting in headache, dizziness, cough, sore throat, nausea, laboured breathing, unconsciousness and death. Inhalation may cause lung oedema. Redness, pain, severe deep burns of eyes.	Extremely flammable; explosive limits 4.3–46%.	Work with ventilation, local exhaust. Wear safety goggles or eye protection in combination with respiratory protection.	Strong oxidizers and strong nitric acid. Attacks many metals and plastics.	Sense of smell becomes rapidly fatigued and cannot be relied on to warn of the continuous presence of the gas.
Iodine I_2	Bluish-black crystalline scales with a characteristic odour; m.p. 114 °C b.p. 184 °C; practically insoluble in water.	Irritation of eyes, respiratory system and skin. Repeated exposure may cause skin sensitization. May have effect on thyroid.	Not combustible but enhances combustion of other substances. Many reactions may cause fire or explosion. Gives off irritating or toxic fumes (or gases) in a fire.	Do not breathe vapour; avoid contact with eyes. Wear nitrile gloves.	Reacts violently with metals including aluminium, potassium and sodium, and with ethanol/phosphorus mixtures, acetylene and ammonia.	

Mercury Hg (Quicksilver)	Heavy silvery liquid; m.p. –39 °C b.p. 357 °C; insoluble in water.	May be absorbed through skin. Repeated exposure may affect kidneys and central nervous system, and may cause vomiting, diarrhoea, headache, nausea, swollen gums, loose teeth.	Not combustible. Gives off irritating or toxic fumes in a fire.	Keep container tightly closed. Work in fume cupboard or well-ventilated area. Prevent spills. Observe strict hygiene. Wear nitrile gloves.	Acetylene, fulminic acid. Reacts with ammonia, azides and ethylene oxide to form explosive products. Reacts violently with bromine. Forms amalgams with many metals.	Store containers and use over catch-ment trays to contain spillage; suck up spilt drop-lets into a small respiratory bottle fitted with a capillary connecting tube and connected to a pump; treat spilt areas with zinc dust to form an amalgam.
Methanol CH_3OH	Colourless volatile liquid with characteristic odour; m.p. –98 °C b.p. 65 °C; miscible with water.	Effects on central nervous system result-ing in unconsciousness; mucous membrane irritation. Chronic exposure can cause damage to retina and optic nerve. Prolonged skin contact may cause dermatitis. May be absorbed through skin.	Highly flammable; flashpoint –16 °C flammable range 7–37%.	Keep container tightly closed; keep away from ignition sources. Avoid breathing vapour and contact with skin. Work in fume cupboard or well-ventilated area. Wear rubber or plastic gloves and eye protection.	Can react vigorously with oxidizers. Reactions with magnesium or bromine can be violent and those with strong oxidants or chloroform with sodium can be explosive.	
Naphthylamine (alpha and beta) $C_{10}H_9N$ *N*-phenyl-α-naphthylamine and *N*-phenyl-β-naphthylamine	White to pink crystals with characteristic odour; alpha: m.p. 50 °C b.p. 301 °C; beta: m.p. 113 °C b.p. 306 °C; poorly soluble in water but hydrochloride is water-soluble.	Both forms very toxic by inhalation, ingestion and skin contact. Human carcinogen causing bladder cancer. Experimental mutagen and teratogen. Absorbed through skin.	Combustible.	Avoid all exposure; wear suitable protective clothing. Work in fume cupboard or hood, or with exhaust ventilation.		Use is prohibited or legally controlled in many countries.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Ninhydrin $C_9H_6O_4$	Pale yellow solid, decomposes before melting at 241 °C. Supplied in aerosol spray cans as 0.5% solution in butanol; soluble in water.	Harmful by ingestion and inhalation. Irritation of eyes, respiratory system and skin. Repeated exposure may cause skin sensitization.	Flammable, combustible solid; flashpoint 39 °C.	Avoid inhalation of the spray or vapour and contact with eyes. Wear rubber or plastic gloves and chemical-grade goggles.		Contact with skin produces a persistent violet stain.
Nitric acid (50–70%) HNO_3	Colourless or pale yellow fuming liquid; m.p. –42 °C b.p. 83–121 °C; miscible in water.	Corrosive; causes severe burns to eyes and skin. Inhalation of vapour may cause lung oedema.	Oxidizer; contact with combustible material may cause fire. Evolves toxic fumes in a fire.	Do not breathe vapour; use respiratory protection. In case of contact with eyes, rinse immediately and seek medical attention; in case of contact with skin, wash off immediately; remove contaminated clothing. Wear PVC gloves, plastic apron and chemical-grade goggles. Work in fume cupboard.	Acetic acid, chromic acid, hydrocyanic acid, aniline, carbon, hydrogen sulfide, bases, metals and many other substances.	Concentrated nitric acid is involved in more dangerous reactions than any other chemical reagent.
Nitrobenzene $C_6H_5NO_2$	Pale yellow oily liquid, with characteristic odour; m.p. 6 °C b.p. 211 °C.	Methaemoglobinaraemia with cyanosis, liver damage; symptoms include blue lips or fingernails, blue skin, dizziness, nausea, weakness, unconsciousness. Absorbed through skin.	Combustible; risk of fire and explosion; flashpoint 88 °C.	Work with ventilation, local exhaust or respiratory protection. Wear protective gloves, protective clothing, safety goggles.	On combustion forms corrosive fumes including nitrogen oxides. Reacts violently with strong oxidants and reducing agents, causing fire and explosion hazard. Attacks many plastics. Forms explosive (thermally unstable) substances or mixtures with many organic and inorganic compounds.	

Osmium tetroxide OsO_4	Pale yellow crystals with pungent odour; m.p. 40 °C b.p. 130 °C; sublimes below boiling point; soluble in water.	Very toxic by inhalation, ingestion and skin contact, causing severe burns and irritation. Vapour, solid and solutions are corrosive to skin and respiratory tract. Inhalation may cause lung oedema.	Powerful oxidizing agent. Not combustible but enhances combustion of other substances.	Keep container tightly closed and in a well-ventilated area. Work with solid and solutions in fume cupboard or hood. Wear chemical-grade goggles and protective gloves. To make up solutions, add unopened ampoule to required volume of water, stopper and shake to break ampoule.	
Oxalic acid HO_2CCO_2H	Colourless crystals; soluble in water; m.p. 190 °C, decomposes.	Harmful if in contact with skin or if ingested. Dust irritates respiratory tract and eyes. Solutions irritate eyes and may cause skin burns.	Combustible. Gives off irritating or toxic fumes (or gases) in a fire.	Avoid contact with skin and eyes; wear eye protection and gloves.	Oxidizing agents; also silver and mercury and their compounds.
Oxygen O_2	Colourless compressed gas; m.p. –218.4 °C b.p. –183 °C.	At very high concentrations, irritation of respiratory tract.	Not combustible but enhances combustion of other substances. Heating will cause rise in pressure of container with risk of bursting.	No open flames, no sparks, no smoking, no contact with flammable substances.	A strong oxidant, reacts with combustible and reducing materials, causing fire and explosion hazard. Reacts with oils, greases, hydrogen, and flammable liquids, solids and gases.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Perchloric acid $HClO_4$	Colourless liquid; miscible with water.	Corrosive; causes severe burns to eyes and skin and if ingested. Vapour is corrosive to eyes, skin, and respiratory tract. Inhalation of vapours may cause lung oedema.	Powerful oxidizing agent. Not combustible but enhances combustion of other substances.	Avoid breathing vapour and other exposure; wear protective clothing including nitrile gloves, eye and face protection. With hot solutions work in fume cupboard or hood.	Combustible materials and reducing agents: acetic anhydride, bismuth and its alloys, alcohol, metal, paper, wood and other organic materials.	Powerful oxidizing agent; may form explosive products if in contact with many inorganic and organic materials; contaminated wooden floors, benches, etc. May explode on percussion.
Phenol C_6H_5OH	Colourless or pale pink crystals with characteristic odour; m.p. 41 °C b.p. 182 °C; soluble in water.	Substance and vapours are corrosive to eyes, skin and respiratory tract causing severe burns; absorbed through skin. Central nervous system disturbance, coma. Kidney and liver damage. Symptoms include abdominal pain, vomiting, diarrhoea, skin irritation, eye pain. Prolonged contact with dilute solutions may cause dermatitis.	Flashpoint 80 °C flammable range 1.7–6%.	Do not breathe vapour; use respiratory protection. Avoid eye and skin contact. Work in fume cupboard. Wear nitrile gloves and eye protection. In case of contact with eyes, rinse immediately with water and seek medical advice; in case of contact with skin, remove any contaminated clothing and swab the contaminated area with glycerol, polyethylene glycol 300 or a mixture of liquid polyethylene glycol (70%) and methylated spirit (30%) and then flush with water.	Reacts with oxidants causing fire and explosion hazard.	

Phosphoric acid H_3PO_4	Colourless viscous liquid or hygroscopic white crystals; m.p. 42 °C decomposes below boiling point at 213 °C; soluble in water.	Corrosive; causes burns to the skin and eyes.	Attacks many metals producing hydrogen. Gives off toxic fumes in a fire.	In case of contact with eyes, rinse with water and obtain medical advice. Wear nitrile gloves and eye protection.		
Phosphorus pentoxide P_2O_5	Hygroscopic white crystals or powder; m.p. 340 °C, sublimation point 360 °C.	Corrosive to the eyes, skin, respiratory tract, leading to sore throat, cough, burning sensation, shortness of breath; skin burns, pain, blisters, eye burns. Inhalation may cause lung oedema. Ingestion may cause abdominal cramps, burning sensation, diarrhoea, sore throat, vomiting.	Not combustible but enhances combustion of other substances. Many reactions may cause fire or explosion. Gives off irritating or toxic fumes (or gases) in a fire.	Work with local exhaust protection. Wear protective gloves and clothing, face shield, or eye protection in combination with respiratory protection.	Solution in water is a strong acid; reacts violently with bases and is corrosive. Reacts violently with perchloric acid causing fire and explosion hazard. Reacts violently with water forming phosphoric acid. Attacks many metals in presence of water.	
Picric acid $C_6H_2(NO_2)_3OH$ 2,4,6-Trinitrophenol	Yellow crystals moistened with water or dissolved in alcohol; m.p. 122 °C; slightly soluble in water.	Toxic by ingestion, inhalation or skin contact. Ingestion may result in headache, nausea. Irritation of eyes.	Explosive when dry.	Keep moistened with water at all times or use only in alcoholic solution.	Forms salts with many metals which are more explosive than the acid itself. In contact with concrete may form calcium picrate, which is a friction-sensitive explosive. May react vigorously with reducing agents.	Yellow skin stains.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Potassium hydroxide KOH	White flakes, powder, pellets or sticks; m.p. 360 °C b.p. 1320 °C; very soluble in water.	Corrosive to respiratory system, eyes and skin; inhalation of dust causes lung oedema.		In case of contact with eyes, rinse immediately with water and seek medical advice; in case of contact with skin, wash immediately; remove contaminated clothing. Wear rubber or plastic gloves and eye protection even for dilute solutions.	Reacts violently with acids and with nitrobenzene and many other detergents. Evolves large quantity of heat when mixed with water; store in a well-sealed container.	Attacks some metals (aluminium, zinc, tin) in the presence of moisture.
Potassium permanganate $KMnO_4$	Purple crystals; m.p. 240 °C (decomposes); readily soluble in water.	Corrosive if swallowed or if dust is inhaled. Extreme irritation of eyes and respiratory tract, inhalation of dust may cause lung oedema.	Powerful oxidizing agent; may ignite combustible materials.	Wear protective clothing, eye protection and particulate respirator if dust is produced.	Reacts violently or explosively if mixed with a wide variety of inorganic and organic compounds or powdered metals.	
Potassium tellurite K_2TeO_3	White deliquescent crystals; very soluble in water.	Toxic by ingestion and inhalation of dust. Irritation of skin and eyes.		Wear protective clothing.		
Propan-2-ol $(CH_3)_2CHOH$ Isopropanol	Colourless liquid with alcoholic odour; m.p. −89 °C b.p 82 °C; miscible with water.	Irritation of eyes and respiratory tract. May affect central nervous system causing headache, dizziness, nausea, vomiting and coma.	Highly flammable; flashpoint 112 °C flammable range 2.3–12.7%.	Keep container tightly closed; keep away from ignition sources. Work in fume cupboard. Wear nitrile gloves and eye protection.	Can react vigorously with oxidizers to form unstable peroxides on prolonged exposure to air and light.	70–85% propan-2-ol in water used as a disinfectant spray remains a flammable hazard and should not be used near ignition sources.

Pyridine C_5H_5N	Colourless liquid with characteristic odour; m.p. 42 °C b.p. 115 °C.	Affects central nervous system causing dizziness, headache, nausea, shortness of breath, unconsciousness. May be absorbed through skin causing redness and burning sensation. Ingestion causes abdominal pain, diarrhoea, vomiting, weakness. Repeated exposure causes liver and kidney effects.	Highly flammable; flashpoint 20 °C explosive limits, 1.8–12.4%. Gives off irritating or toxic fumes (or gases) in a fire. Vapour/ mixtures are explosive.	Work with ventilation, local exhaust or respiratory protection; wear gloves and protective clothing.	Reacts violently with strong oxidants and strong acids.
Selenium Se	Odourless solid in various forms, dark red-brown to bluish-black amorphous solid or red transparent crystals or metallic grey to black crystals; m.p. 170–217 °C b.p. 685 °C.	Irritation of skin and eye. Inhalation of dust may cause lung oedema. Repeated exposure may cause loss of nails, gastrointestinal effects.	Combustible. Gives off irritating or toxic fumes (or gases) in a fire.	Prevent dispersion of dust. Observe strict hygiene. Work with local exhaust. Wear protective gloves, clothing, and safety spectacles.	Reacts violently with oxidants and strong acids. Reacts with water at 50 °C forming flammable hydrogen and selenious acids. Reacts with incandescence on gentle heating with phosphorus and metals such as nickel, potassium, platinum, sodium and zinc.
Silver Ag	White metal, turns dark on exposure to ozone, hydrogen sulfide or sulfur; m.p. 962 °C b.p. 2212 °C.	Inhalation of high amounts of metallic silver vapours may cause lung damage with pulmonary oedema. May cause a grey-blue discoloration of the eyes, nose, throat and skin on long-term or repeated exposure (argyria).	Not combustible except as powder.	Work with local exhaust. Wear protective gloves and safety spectacles or eye protection in combination with respiratory protection for powder or fume.	Incompatible with acetylene, ammonium compounds, oxalic acid and tartaric acid.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Silver nitrate $AgNO_3$	White crystals; m.p. 212 °C b.p. 444 °C; soluble in water.	May cause severe irritation and burns to eyes and skin. Corrosive by ingestion. May cause a red-blue discoloration of the skin on long-term or repeated exposure (argyria).	Not combustible but enhances combustion of other substances.	Prevent dispersion of dust. Observe strict hygiene. Wear protective rubber or plastic gloves, and face shield or eye protection in combination with respiratory protection. In case of contact with eyes, rinse with water and seek medical advice.	Ammoniacal solutions can precipitate explosive silver nitrite in the presence of base or glucose. Can form explosive products with ethanol and may cause explosive polymerization with acrylonitrile. May cause ignition of explosion if mixed with charcoal, magnesium, phosphorus or sulfur.	
Sodium azide N_3Na	Colourless crystalline solid; m.p. 300 °C; soluble in water.	Very toxic by ingestion, inhalation and skin contact; may cause burns. Dust and solution irritate eyes and skin: may be absorbed through skin.	Decomposes explosively when heated above its melting point. Gives off toxic fumes when heated: do not use water to extinguish fires.	In case of contact with skin, wash immediately. Do not inhale dust. Wear rubber or plastic gloves and eye protection.	Explosive reactions with bromine, carbon disulfide or chromyl chloride. Solid reacts with heavy metals including copper, lead and mercury to form explosive metal azide salts. On contact with acid, develops highly toxic and explosive gas.	
Sodium biselenite $NaHSeO_3$	Colourless, white crystalline powder; soluble in water.	Toxic by ingestion and inhalation of dust; possible danger of cumulative effects. Experimental teratogen. Prolonged skin contact may cause dermatitis.		Wear protective clothing.	Oxidizing agents.	

Sodium cyanide NaCN	White crystalline powder with almond odour; m.p. 563 °C b.p. 1496 °C; very soluble in water.	Extremely toxic by ingestion, inhalation and skin contact; severely irritating to eyes. May be absorbed through skin. Repeated exposure may affect thyroid.	May give off toxic fumes in a fire.	Do not inhale dust; use respiratory protection. Avoid eye and skin contact; in case of contact with skin, wash immediately with water and remove contaminated clothing. Wear chemical-grade goggles and rubber or plastic gloves. Keep in a securely locked, ventilated store.	Liberates extremely toxic hydrogen cyanide (HCN) gas on contact with acids or with water containing dissolved carbon dioxide. Can form explosive mixtures with nitrites.	Treat spillage of solutions with bleaching powder (sodium hypochlorite) and leave for 24 h. Sweep up solid spills carefully and add to water containing bleaching powder; leave for 24 h before discarding. Keep cyanide antidote kit available in the laboratory.
Sodium hydroxide NaOH	Colourless flakes, powder, pellets or sticks; m.p. 318 °C b.p. 1390 °C; soluble in water.	Solid and concentrated solute. Inhalation of dust causes damage to respiratory tract, lung oedema. Corrosive by ingestion. Dilute solutions irritating to eyes or may cause severe damage if eye contact is prolonged.	Not combustible. Contact with moisture or water may generate sufficient heat to ignite combustible substances.	In case of contact with eyes rinse immediately and seek medical advice; in case of contact with skin wash immediately with water, remove contaminated clothing. Wear rubber or plastic gloves and eye protection even with dilute solutions.	Evolves large quantity of heat when mixed with water. Reacts vigorously with chloroform-methanol mixtures and with strong acids.	Store in well-sealed container in dry place.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Sodium hypochlorite solution (10–14% available chlorine) NaOCl	Colourless or pale yellow solution with chlorine odour; miscible with water.	Corrosive to eyes and skin; corrosive by ingestion and to respiratory tract; inhalation may cause lung oedema. Repeated exposure may cause skin sensitization.	Strong oxidant. May give off toxic fumes in a fire.	In case of contact with eyes, rinse immediately with water and seek medical advice; in case of contact with skin, wash immediately. Do not inhale vapour; use respiratory protection. Work in well-ventilated area. Wear rubber or plastic gloves and chemical-grade eye protection.	Liberates highly toxic gas in contact with acids. Can react vigorously with combustible and reducing compounds. May react with nitrogen compounds to form explosive N-chloro-compounds; may react violently with methanol.	Gradually loses chlorine during storage; dilute solutions used as disinfectant rapidly deteriorate. Store away from acids in a dark, cool, well-ventilated area.
Sulfuric acid H_2SO_4	Colourless, odourless viscous liquid; m.p. 10°C b.p. (decomposes) 340 °C.	Concentrated solution (15%) corrosive, causes severe burns; mist and vapour highly corrosive by inhalation; dilute solutions irritating to eyes and skin; cause burns and dermatitis.	May give off toxic fumes in a fire. Not combustible. Many reactions may cause fire or explosion. Dilution with water generates heat and spattering or boiling may occur. Always add acid to water, **never add water to acid.**	In case of contact with eyes rinse immediately and seek medical advice; in case of contact with skin wash immediately remove contaminated clothing. Wear nitrile gloves, eye and face protection. **No contact with flammable substances.**	Is a powerful oxidizing desiccant and reacts violently with many reagents including organic nitro compounds, potassium permanganate, alkali metals and per-chlorates, combustible materials, oxidizers, amines, bases, water, excess heat and most metals.	Localized boiling may occur if concentrated acid is added to water.

Tetrahydrofuran C_4H_8O Diethylene oxide Tetramethylene oxide	Colourless liquid, with characteristic odour; m.p. –108.5 °C b.p. 66 °C.	Central nervous system depressant causing narcosis. Eye, skin and respiratory irritation.	Highly flammable; may form explosive peroxides; flashpoint –14 °C. Water may be ineffective to fight fires involving tetrahydrofuran, but it can be used to cool fire-exposed containers.	Work with ventilation, local exhaust or respiratory protection, protective gloves, safety spectacles.	Reacts violently with strong oxidants, strong bases and some metal halide, causing fire and explosion hazard. Attacks some forms of plastics, rubber and coatings. Tetrahydrofuran may polymerize in the presence of cationic initiators. Refluxing with calcium hydroxide can cause explosions.
Thallium acetate $TlC_2H_3O_2$	White deliquescent crystals; m.p. 110 °C; very soluble in water.	Extremely toxic by ingestion with possible cumulative effects. Affects nervous and cardiovascular systems. Harmful through eye and skin contact.		Keep container tightly closed. Work in fume cupboard, hood or with exhaust ventilation. Wear protective clothing including dust respirator, chemical-grade goggles, rubber or plastic gloves, eye protection.	
o-Tolidine $(C_6H_3\text{-}(3CH_3)\text{-}(4NH_2))_2$ 3,3'-Dimethyl-(1,1'-biphenyl)-4,4'-diamine	Colourless crystals; m.p. 131 °C b.p. 200 °C; poorly soluble in water.	Harmful by contact with skin or ingestion. Dust irritates respiratory tract and eyes. Probably a human carcinogen.	Combustible. Gives off irritating or toxic fumes (or gases) in a fire.	Avoid contact; wear eye protection and gloves.	Oxidizing agents.

CHEMICAL	PHYSICAL PROPERTIES	HEALTH HAZARDS	FIRE HAZARDS	SAFETY PRECAUTIONS	INCOMPATIBLE CHEMICALS	OTHER HAZARDS
Toluene C_7H_8 Methylbenzene	Colourless liquid with characteristic odour; m.p. –95 °C b.p. 111 °C; not miscible with water.	Central nervous system depressant. Irritation of eyes, mucous membranes, skin. Repeated exposure may cause toxicity in human reproduction or development.	Highly flammable; vapour may cause flash fire; flashpoint 4 °C flammable range 1.4–7%. Extinguishing media for a small fire: dry chemicals, carbon dioxide, foam, water fog or inert gas (nitrogen).	Keep container tightly closed; keep away from ignition sources; earth/(ground) containers to prevent static electrical discharge. Do not inhale vapour; use respiratory protection. Work in fume cupboard or well-ventilated area. Wear nitrile gloves.	Can react with strong acids, alkalis and oxidizers.	
Trichloroacetic acid CCl_3COOH	White hygroscopic crystals with pungent odour; m.p. 58 °C b.p. 197.5 °C; soluble in water, ethanol, diethylether.	Corrosive; causes severe burns to eyes, skin, respiratory tract.	Not combustible. May give off toxic fumes in fire.	Avoid contact with eyes and skin; wear rubber or plastic gloves and chemical-grade goggles or face shield in combination with respiratory protection. In case of contact with eyes, rinse immediately and seek medical advice.	Violent reaction with copper/dimethyl sulfoxide mixtures and on contact with bases, strong oxidizing agents and metals such as iron, zinc, aluminium.	Store in a dry place. Concentrated aqueous solutions may decompose violently.

Trichloro-ethylene $CHClCCl_2$	Colourless liquid, characteristic odour; m.p. –73 °C b.p. 87 °C.	Irritation of eyes, skin; prolonged exposure may cause dermatitis and affect the central nervous system resulting in loss of memory. May affect liver and kidneys. Probably a human carcinogen.	Combustible under specific conditions.	Work with ventilation, local exhaust. Wear gloves, safety spectacles or other eye protection in combination with respiratory protection.	On contact with hot surfaces or flames, decomposes forming toxic and corrosive gases (phosgene, hydrogen chloride). Decomposes on contact with strong alkali producing dichloro-acetylene; reacts violently with metal powders such as aluminium, barium, magnesium and titanium; slowly decomposed by light in the presence of moisture, with formation of hydrochloric acid.	
Xylene (mixed isomers) $C_6H_4(CH_3)_2$ Dimethyl-benzene	Colourless liquid with aromatic odour; m.p. –95 to –13 °C b.p. 136–145 °C; insoluble in water.	May affect central nervous system resulting in headache, dizziness, fatigue and nausea. Liquid and vapour irritate eyes, skin, mucous membranes, respiratory tract. Harmful if ingested. Prolonged skin contact may defat the skin. Non-specific neurological impairment. Exposure may enhance hearing damage caused by exposure to noise. Animal tests suggest toxicity to human repro-duction or development.	Flammable liquid; flashpoint 27–32 °C.	Avoid contact with eyes. Wear nitrile gloves and eye protection. Keep container tightly closed; keep away from ignition sources.		May contain ethylbenzene as an impurity. Ethylbenzene is a possible human carcinogen.

ANNEX 6 – Biomedical Research Acronyms

A1HV-1	Alcelaphine Herpesvirus-1
ABSA	ABSA International
ABSL	Animal Biosafety Level
ACAV	American Committee on Arthropod-Borne Viruses
ACIP	Advisory Committee on Immunization Practices - USA
ACG	Arthropod Containment Guidelines
ACL	Arthropod Containment Levels
ACME	American Committee of Medical Entomology
AHS	African Horse Sickness
AHSV	African Horse Sickness Virus
AKAV	Akabane Virus
ANSI	American National Standards Institute
APHIS	Animal and Plant Health Inspection Service - USA
APMV-1	Avian Paramyxovirus Type 1
ASF	African Swine Fever
ASFV	African Swine Fever Virus
ASHRAE	American Society of Heating, Refrigerating, and Air-Conditioning Engineers
ASTMH	American Society of Tropical Medicine and Hygiene
BCG	Bacillus Calmette-Guérin
BDV	Border Disease Virus
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BoNT	Botulinium neurotoxin

BSC	Biosafety Cabinet
BSE	Bovine Spongiform Encephalopathy
BSL	Biosafety Level
BSL-3-Ag	BSL-3-Agriculture
BSO	Biosafety Officer
BTV	Bluetongue Virus
BVDL	Bovine Viral Diarrhea Virus
cGLP	Current Good Laboratory Practice - FDA
cGMP	Current Good Manufacturing Practice - FDA
CAV	Constant Air Volume
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
CETBE	Central European Tick-Borne Encephalitis
CDC	Centers for Disease Control and Prevention
CHV-1	Cercopithecine Herpesvirus-1
Ci	Curie Radiation Unit
CFM	Cubic Feet per Minute
CJD	Creutzfeldt-Jakob Disease
CJIS	Criminal Justice Information Services Division - USA
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CSFV	Classical Swine Fever Virus
DHHS	Department of Health and Human Services - USA
DoC	Department of Commerce - USA
DOD	Department of Defense - USA
DOL	Department of Labor - USA

DOT	Department of Transportation - USA
EBV	Epstein-Barr Virus
EEE	Eastern Equine Encephalomyelitis
EPA	Environmental Protection Agency - USA
EtOH	Ethanol
FDA	Food and Drug Administration - USA
FFI	Fatal Familial Insomnia
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GHS	Globally Harmonized System of Classification & Labeling
GI	Gastrointestinal Tract
GMO	Genetically Modified Organism
GSS	Gerstmann-Straussler-Scheinker Syndrome
HEPA	High Efficiency Particulate Air
HBV	Hepatitis B Virus
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HD	Heartwater Disease
HDV	Hepatitis D Virus
HFRS	Hemorrhagic Fever with Renal Syndrome
HHV	Human Herpes Virus
HHV-6A	Human Herpes Virus -6A
HHV-6B	Human Herpes Virus -6B
HHV-7	Human Herpes Virus -7
HHV-8	Human Herpes Virus -8
HIV	Human Immunodeficiency Virus

HPAI	Highly Pathogenic Avian Influenza
HPAIV	Highly Pathogenic Avian Influenza Virus
HPS	Hantavirus Pulmonary Syndrome
HSV-1	Herpes Simplex Virus-1
HSV-2	Herpes Simplex Virus-2
HTLV	Human T-Lymphotropic Viruses
HVAC	Heating, Ventilation, and Air Conditioning
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICAO	International Civil Aviation Organization
ID	Infectious Dose
ID50	Number of organisms necessary to infect 50% of a group of animals
IEST	Institute of Environmental Sciences & Technology
IgG	Immunoglobulin
ILAR	Institute for Laboratory Animal Research
IND	Investigational New Drug
IPM	Integrated Pest Management
IPV	Inactivated Poliovirus Vaccine
ISA	Infectious Salmon Anemia
ISAV	Infectious Salmon Anemia Virus
ISO	International Organization for Standardization
LAI	Laboratory-Associated Infections
LAN	Local Area Network
LCM	Lymphocytic Choriomeningitis
LCMV	Lymphocytic Choriomeningitis Virus

LD	Lethal Dose
LED	Light Emitting Diode
lfm	Linear Feet Per Minute
lm/s	Linear Meters Per Second
LGV	Lymphogranuloma Venereum
LMW	Low Molecular Weight
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
MCF	Malignant Catarrhal Fever
MenV	Menangle Virus
MERS CoV	Middle East Respiratory Syndrome
MMWR	Morbidity and Mortality Weekly Report
MOPH	Ministry of Public Health
MPPS	Most Penetrating Particle Size
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
NBL	National Biocontainment Laboratory
NCI	National Cancer Institute - USA
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NHP	Nonhuman Primate
NIH	National Institutes of Health - USA
NIOSH	National Institute for Occupational Safety and Health– USA
NSF	NSF International
OBA	NIH Office of Biotechnology Activities
OIE	World Organization for Animal Health

OPV Oral Poliovirus Vaccine

OSHA Occupational Safety and Health Administration

OSP NIH Office of Science Policy

Pa Pascal Unit of Pressure

PAPR Positive Air-Purifying Respirator

PBT Pentavalent Botulinum Toxoid Vaccine

PPD Purified Protein Derivative

PPE Personal Protective Equipment

PPM Parts Per Million

PPRV Pest des Pesits Ruminants Virus

Prp Prion Protein

PSDS Pathogen Safety Data Sheet – Canada

PVC Polyvinyl chloride

PTFE Polytetrafluoroethylene

RAC Recombinant DNA Advisory Committee

RBL Regional Biocontainment Laboratory

RG Risk Group

RP Rinderpest

RPV Rinderpest Virus

RVF Rift Valley Fever

RVFV Rift Valley Fever Virus

SALS Subcommittee on Arbovirus Laboratory Safety

SARS Severe Acute Respiratory Syndrome

SARS-CoVSARS-Associated Coronavirus

SCID Severe Combined Immune Deficient

SC type Small-Colony type

SE Staphylococcal Enterotoxins

SEA SE Serotype A

SEB SE Serotype B

SIV Simian Immunodeficiency Virus

SGP Sheep and Goat Pox

SGPV Sheep and Goat Pox Virus

SMP Standard Microbiological Practice

SOP Standard Operating Procedure

SVCV Spring Viremia of Carp Virus

Sv Sievert Radiation Dose

- Definition - External dose quantities... Calculating protection dose

SVD Swine Vesicular Disease

SVDV Swine Vesicular Disease Virus

TLV Threshold Limit Values

TME Transmissible Mink Encephalopathy

TSE Transmissible Spongiform Encephalopathy

ULPA Ultra Low Penetrating Air

USAMRIIDU.S. Army Medical Research Institute of Infectious Diseases

USDA U.S. Department of Agriculture

USPS United States Postal Service

UPS Uninterrupted Power Supply

UV Ultraviolet Radiation

VAV Variable Air Volume

VEE Venezuelan Equine Encephalitis

VS Veterinary Services

VZV Varicella-Zoster Virus

WEE Western Equine Encephalomyelitis

WHO World Health Organization

Wi-Fi Wireless Fidelity, Wireless Internet

WNV West Nile Virus