UNIVERSITY of DENVER

BIOSAFETY MANUAL

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Introduction

DU BIOSAFETY MANUAL

It is the goal of the University of Denver (DU) to provide a safe environment for students, faculty, staff, and the environment. The guidelines and recommendations indicated in this manual will help ensure the safety of personnel and the workplace as it relates to biological materials.

I. Scope

These policies are for all investigators and staff who conduct, or are engaged in biological or biomedical studies at DU.

A. These policies are intended for work that involves the use of recombinant DNA, human blood/tissues, animal blood/tissues, and other potentially infectious agents.

B. The policies will address compliance with the NIH Guidelines for Research Involving recombinant DNA, OSHA Bloodborne Pathogens Standard, the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, and the OSHA Laboratory Standard and the Hazard Communication Standard, as applicable.

C. Good laboratory practices and standard precautions should be practiced in any laboratory where biomedical research is being conducted.

This document is written as a guideline for work in the research. It addresses engineering controls, work practices and personal protective equipment that should be implemented to minimize the exposure to infectious agents or other potentially infectious material, recombinant DNA, or material and/or its toxin that is known or suspected to cause human or animal disease or harm.

Researchers conducting experiments at DU are required to follow specific guidelines which are adapted from federal, state, and local agencies. The most effective means to minimize potential hazards associated with recombinant DNA and infectious agents is to combine professional experience with carefully performed laboratory procedures.

Contact:
Institutional Biosafety Committee – 303-871-2121
IBCAdmin@du.edu

Experimental protocols shall be submitted to the DU Institutional Biosafety Committee (IBC), via IRBNet, https://irbnet.org.

1. BIOSAFETY GUIDELINES AND STANDARDS

The CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, the OSHA Occupational Exposure to Bloodborne Pathogens Standard, and the NIH Guidelines involving Research with Recombinant DNA Molecules are most often referred to for the biomedical research that is conducted at DU.

1.1 CDC/NIH Biosafety in the Microbiological and Biomedical Laboratories

The CDC/NIH "Biosafety in the Microbiological and Biomedical Laboratories" guidelines review the principles of biosafety including laboratory practices and techniques to be used in the biological laboratory conducting experiments with infectious agents and other potentially infectious materials. Agent summary listing and statements are provided, including description of practices to be followed for the more common infectious agents. The listing is categorized per the class of infectious agent, i.e. parasitic, fungal, bacterial/rickettsial, and viral. Safety equipment, facility design, biosafety levels and import and interstate shipment of biomedical materials are addressed. Practices and techniques to be followed at these biosafety levels are described. Animal biosafety levels are also described. The biosafety level classifications are described in Section 3. Guidelines for shipping specimens and infectious materials may be found in Section VI.

1.2 OSHA Occupational Exposure to Bloodborne Pathogens Standard Title 29, CFR Part 1910.1030

The OSHA Occupational Exposure to Bloodborne Pathogens Standard, necessitates that DU provide and adhere to a written policy concerning the handling of such materials.

Per the OSHA standard, DU employees are evaluated for the potential for exposure to bloodborne pathogens. All employees at risk are provided training concerning the hazards, recognition of tasks which would lead to exposure, how to use personal protective equipment and engineering controls to minimize or eliminate this exposure, epidemiology regarding HIV, Hepatitis B, Hepatitis C, Hepatitis B vaccination information, as well as incident reporting and incident required follow-up.

In accordance with the OSHA BBP Standard shall be available in any work area where there is a potential of exposure. The materials that can lead to exposure to bloodborne pathogens are identified below. This list identifies general categories of infectious materials most commonly associated with bloodborne pathogens.

Human blood and other potentially infectious materials are defined as follows:

- Human blood, human blood components, products made from human blood
- Other Potentially Infectious Materials (OPIM), including:

  Human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids where it is difficult to differentiate if the fluid is contaminated.

  Any unfixed human tissue or organ, other than intact skin, from a human - living or dead.

  Materials containing HIV, HBV, and HCV: cell or tissue cultures, organ cultures and HIV, HBV, HCV - containing culture medium or other solutions; and blood, organs, and/or other tissues from experimental animals infected with HIV, HBV, and HCV.

If you have questions relating to bloodborne pathogens and/or risk associated with occupational exposure, please the IBC at 1-2121.

1.3. NIH Guidelines for Research Involving Recombinant DNA Materials

The NIH Guidelines should be followed whenever research protocols involve the use of recombinant DNA materials. This document provides information regarding the protocol approval process and methods to assist research investigators to perform experiments in a safe manner to protect the worker and minimize the risk of exposure to potentially biohazardous materials. The guidelines identify what types of experiments are exempt as well as specific directions and protocol approval requirements necessary for specific categories of experiments. The approval process begins with the review of the projects involving recombinant DNA molecules by the DU Institutional Biosafety Committee (IBC). Also included in the NIH recombinant DNA Guidelines are recommendations for physical and biological containment, shipment of materials, classification of infectious agents, and biosafety level descriptions for animals, plants, and infectious agents.

This summary of the guidelines should be used only as a reference and should be used in addition to the full guidelines as published by the NIH/CDC. The guidelines should be referred to for specific definitions and clarifications. If further questions require resolution, these can be referred to the IBC. A current listing of infectious agents and their appropriate biosafety level, as determined by the NIH and CDC are included in Section 2.

Experimental protocols shall be submitted to the DU Institutional Biosafety Committee (IBC), via IRBNet, https://irbnet.org. (Call 303-871-2121 for assistance).

Summary of the NIH Guidelines for Research Involving Recombinant DNA Molecules

Exempt Experiments
Experiments are **exempt** from NIH recombinant DNA Guidelines if:

a) they do not include the use of organisms or viruses (ex. PCR, Northern or Southern blotting),

b) they involve DNA segments consisting entirely of single non-chromosomal source or manipulations of a viral DNA source with the intentional introduction of a deletion or mutation into a viral genome involving no foreign DNA,

c) they consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses (propagated in that host or transferred by well-known physiological means) (ex. E. Coli),

d) they consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (excluding viruses) propagated in that host (ex. transformation of human cells with human DNA),

e) they consist entirely of DNA from different species that exchange DNA by well-known physiological processes and no human pathogen is involved. (ex., Salmonella and E. coli), and

f) the rDNA molecules used in the protocols contain <1/2 of any eukaryotic viral genome, are propagated in E. coli K12, Saccharomyces, Bacillus subtillis or B. lichenformis host-vector system, or are recombinant DNA molecules extrachromosomal elements of Gram positive organisms as listed in Appendix C- V of the NIH Guidelines, and are not included in a higher level category requiring additional approvals (i.e. the experiments do not include DNA from Class 3, 4, or 5 organisms, are not large scale experiments (less than 10 Liters of culture), or do not include the cloning of a toxin molecule gene where gene product (s) is (are) toxic to vertebrates.)

The following experiments require IBC notice simultaneous with initiation:
Experiments not included in Sections III-A, III-B, III-C, III-D, III-F, and their subsections are considered in Section III-E (of NIH Guidelines). All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III-D, Experiments that Require Institutional Biosafety Committee Approval before Initiation) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but

Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV-A, Policy). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-E and may be conducted at BL1 containment.

The following experiments require only IBC approval prior to initiation:

1) Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See Section II-A, Risk Assessment)
2) Experiments in Which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems
3) Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems
4) Experiments Involving Whole Animals
5) Experiments Involving Whole Plants
6) Experiments Involving More than 10 Liters of Culture
7) Experiments Involving Influenza Viruses

The following experiments require IBC and NIH approval:

1) Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms per Kilogram Body Weight
2) Experiments that have been Approved (under Section III-A-1-a) as Major Actions under the NIH Guidelines

The following experiments require IBC and RAC Review (if applicable) Before Research Participant Enrollment:

1) Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Specific Exemptions Defined

EXEMPTIONS UNDER SECTION III-F-8

Section III-F-8 states that exempt from these *NIH Guidelines* are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Sections III-F-8*, for other classes of experiments which are exempt from the *NIH Guidelines*." The following classes of experiments are exempt under Section III-F-8:

Appendix C-I. Recombinant or Synthetic Nucleic Acid Molecules in Tissue Culture

Recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see Appendix C-IX-E,

Footnotes and References of Appendix C), that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed in Appendix C-I-A.

Appendix C-I-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH OSP and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents, (iii) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*), and (iv) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

Appendix C-II. *Escherichia coli K-12* Host-Vector Systems

Experiments which use *Escherichia coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the *NIH Guidelines* provided that: (i) the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-IX-B, *Footnotes and References of Appendix C*) shall be used as vectors. However, experiments involving the insertion into *Escherichia coli* K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-IX-C, *Footnotes and References of Appendix C*) with *Escherichia coli* may be performed with any *Escherichia coli* K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *Escherichia coli* K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant or synthetic nucleic acid molecule techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-II-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH OSP and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human*

Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, *Footnotes and References of Sections I*
through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-III. *Saccharomyces* Host-Vector Systems

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the *NIH Guidelines*. For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the unmodified host organism; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-III-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH OSP and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-IV. *Kluyveromyces* **Host-Vector Systems**

Experiments involving *Kluyveromyces lactis* host-vector systems, with the exception of experiments listed in Appendix C-IV-A, are exempt from the *NIH Guidelines* provided laboratory-adapted strains are used (i.e. strains that have been adapted to growth under optimal or defined laboratory conditions). For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the unmodified host organism; the Institutional Biosafety Committee may specify higher containment if deemed necessary.

Appendix C-IV-A Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B, which require NIH OSP and Institutional Biosafety Committee approval before initiation; (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval; (iii) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-V. *Bacillus subtilis* **or** *Bacillus licheniformis* **Host-Vector Systems**

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than 10^{-7} may be used for cloning DNA with the exception of those experiments listed in Appendix C-V-A, *Exceptions*. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the unmodified host organism; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-V-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH OSP and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-VI. Extrachromosomal Elements of Gram Positive Organisms

Recombinant or synthetic nucleic acid molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these *NIH Guidelines*.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylococcus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes

Streptococcus salivarius
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophilus

Appendix C-VI-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH OSP and Institutional Biosafety Committee approval before

initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-VII. The Purchase or Transfer of Transgenic Rodents

The purchase or transfer of transgenic rodents for experiments that require BL1 containment (See Appendix G-III-M, *Footnotes and References of Appendix G*) are exempt from the *NIH Guidelines*.

Appendix C-VIII. Generation of BL1 Transgenic Rodents via Breeding

The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the *NIH Guidelines* if:
(1) Both parental rodents can be housed under BL1 containment; and
(2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
(3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

Appendix C-IX. Footnotes and References of Appendix C

Appendix C-IX-A. The NIH Director may revise the classification for the purposes of these *NIH Guidelines* (see Section IV-C-1-b-(2)-(b), *Minor Actions*). The revised list of organisms in each Risk Group is located in Appendix B.

Appendix C-IX-B. A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

Appendix C-IX-C. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III-F-6, *Exempt Experiments*.

Appendix C-IX-D. As classified in the *Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses*, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

Appendix C-IX-E. i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

2. BIOHAZARDOUS MATERIALS

Biomedical research conducted at DU often involves experimentation and manipulation of various types of potentially infectious systems. These can be segregated into three general categories:

Human Blood and other potentially infectious materials

Any work which involves experimentation or manipulations of human blood or other materials of human origin, may place the worker at risk of exposure to bloodborne pathogens found in them. Some examples of work which would result in exposure to these would be in a clinical laboratory performing tests or analyses on human blood or other potentially infectious materials, research labs performing experiments and/or manipulations with human blood or unfixed tissues or organs, or a maintenance worker repairing a sewer line contaminated with human blood or other potentially infectious materials.

Infectious Agents and Materials

Infectious agents or pathogens are disease-causing microorganisms. There are hundreds of microorganisms which cause disease in man and animals, which globally account for half of all human diseases. Pathogens are classified as bacteria, fungi, viruses, parasites, oncogenic viruses, and prions. A list of pathogens with their respective biosafety levels are included in this section.

Any materials that come in contact with infectious agents and their by-products must be handled as infectious agents themselves. The practices and techniques that are followed are of the greatest importance in minimizing or eliminating occupational exposure to infectious agents. Infectious agents are classified as to their infectivity and viability. These classifications are referred to as the biosafety levels. The biosafety levels are specified by the CDC (Centers for Disease Control and Prevention), the NIH (National Institute of Health), and the World Health Organization (WHO).

Recombinant DNA Molecules and Products

Some of the research performed at DU involves experiments with recombinant DNA materials. Commonly used host-vector systems use E. coli as the host. Regardless of the cloning method utilized, precautions must be taken to assure that the sterility of the product, organism or culture is maintained, as well as assuring that the systems do not cause disease in the operator. It is important to understand what class of agents is being used and that the appropriate facilities and containment equipment are available and used. The NIH Research Involving Recombinant DNA Molecules Guidelines are very specific concerning these issues.

Miscellaneous Biohazardous Materials

There are materials which research staff are often in contact with which warrant caution as they affect individuals differently. These materials include allergens, cultured animal cells and their potentially infectious agents, tissues from experimental animals (including animal dander), plant viruses, bacteria and fungi, and toxins (bacterial, plant, etc.).

Biohazardous Agents by Category

Biohazardous agents have been classified on the basis of their potential hazard. These classifications are also used by the NIH and CDC. The agents must be handled in accordance with the Guidelines as is appropriate for the Class of the agent. The proper handling is detailed in Section 2, entitled “Biosafety Levels and Containment".

The general categories are as follows:

- Bacteria
  - a) Bacterial pathogens
  - b) Bacterial with drug resistance plasmids
- Fungi
- Viruses, viroids, and prions
  - a) Oncogenic viruses
  - b) Other animal viruses
- Rickettsiae
- Chlamydiae
- Parasites
- Recombinant DNA and products
- Some Algae
- All clinical specimens (tissue, fluids, etc.)

2.1 Classification of Biohazardous Agents on the Basis of Hazard

Here is a really useful resource that categorizes biological agents:
Biological Agents – BSL/Risk Group/Pathogen/Select Agent File
(Thanks to the University of Pennsylvania)

Risk Group 1 Agents

- All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes shall be considered Class 1 agents.

Risk Group 2 Agents

- **Risk Group 2 Bacterial Agents (including Chlamydia)**

Acinetobacter baumannii (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3))
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
--*Clostridium botulinum*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
--*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--**Francisella tularensis* specifically **F. tularensis* subspecies *novicida* [aka *F. novicida*], strain Utah 112; **F. tularensis* subspecies *holarctica* LVS; **F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)
*For research involving high concentrations, BL3 practices should be considered (see Appendix G-II-C-2. Special Practices (BL3)).
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonae, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M.*

paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoeae*, *N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Pseudomonas aeruginosa*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. choleraesuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahaemolyticus, V. vulnificus*
--*Yersinia enterocolitica*
--*Yersinia pestis* specifically *pgm*$^{(-)}$ strains (lacking the 102 kb pigmentation locus) and *lcr*$^{(-)}$ strains (lacking the LCR plasmid)

- **Risk Group 2 Fungal Agents**

--*Blastomyces dermatitidis*
--*Cladosporium bantianum, C. (Xylohypha) trichoides*
--*Cryptococcus neoformans*
--*Dactylaria galopava (Ochroconis gallopavum)*
--*Epidermophyton*
--*Exophiala (Wangiella) dermatitidis*
--*Fonsecaea pedrosoi*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*
--*Sporothrix schenckii*
--*Trichophyton*

- **Risk Group 2 Parasitic Agents**

--*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
--*Ascaris* including *Ascaris lumbricoides suum*
--*Babesia* including *B. divergens, B. microti*
--*Brugia* filaria worms including *B. malayi, B. timori*
--*Coccidia*
--*Cryptosporidium* including *C. parvum*
--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
--*Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
--*Entamoeba histolytica*
--*Enterobius*
--*Fasciola* including *F. gigantica, F. hepatica*
--*Giardia* including *G. lamblia*
--*Heterophyes*
--*Hymenolepis* including *H. diminuta, H. nana*

--Isospora
--Leishmania including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruviana, L. tropica*
--Loa loa filaria worms
--Microsporidium
--Naegleria fowleri
--Necator human hookworms including *N. americanus*
--Onchocerca filaria worms including, *O. volvulus*
--Plasmodium including simian species, *P. cynomolgi, P. falciparum, P. malariae, P. ovale, P. vivax*
--Sarcocystis including *S. sui hominis*
--Schistosoma including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
--Strongyloides including *S. stercoralis*
--Taenia solium
--Toxocara including *T. canis*
--Toxoplasma including *T. gondii*
--Trichinella spiralis
--Trypanosoma including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--Wuchereria bancrofti filaria worms

- **Risk Group 2 Viruses**

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses
--Chikungunya vaccine strain 181/25
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
--Western equine encephalomyelitis virus

Arenaviruses
--Junin virus candid #1 vaccine strain
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Caliciviruses

Coronaviruses

Flaviviruses - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Japanese encephalitis virus strain SA 14-14-2
--Yellow fever virus vaccine strain 17D
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)
--Cytomegalovirus
--Epstein Barr virus
--Herpes simplex types 1 and 2
--Herpes zoster
--Human herpesvirus types 6 and 7

Orthomyxoviruses
--Influenza viruses types A, B, and C (except those listed in Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*)
--Tick-borne orthomyxoviruses

Papilloma viruses
--All human papilloma viruses

Paramyxoviruses
--Newcastle disease virus
--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus

Parvoviruses
--Human parvovirus (B19)

Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types
--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses
--Rabies virus - all strains
--Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (*e.g.* Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (*e.g.* Ogden, Hazelhurst)

Rubivirus (Togaviruses)
--Rubella virus

Risk Group 3 Agents

- **Risk Group 3 Bacterial Agents including Rickettsia**

--Bartonella
--Brucella including *B. abortus, B. canis*, *B. suis*
--Burkholderia (Pseudomonas) mallei, B. pseudomallei
*--Coxiella burnetii (*except the Phase II, Nine Mile strain listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
*--Francisella tularensis (*except those strains listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
--Mycobacterium bovis (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
--Orientia tsutsugamushi (was *R. tsutsugamushi*)
--Pasteurella multocida type B -"buffalo" and other virulent strains
--Rickettsia akari, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R, siberica*, *R. typhi* (*R. mooseri*)
*--Yersinia pestis (*except those strains listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*

- **Risk Group 3 Fungal Agents**

--Coccidioides immitis (sporulating cultures; contaminated soil)
--Histoplasma capsulatum, H. capsulatum var. duboisii

- **Risk Group 3 Parasitic Agents**
  - None

- **Risk Group 3 Viruses and Prions**

Alphaviruses (Togaviruses) - Group A Arboviruses
--Chikungunya virus (except the vaccine strain 181/25 listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
--Semliki Forest virus
--St. Louis encephalitis virus

--Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see Appendix B-II-D (RG2) – Viruses)
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Arenaviruses
--Flexal
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus

Coronaviruses
--SARS-associated coronavirus (SARS-CoV)
--Middle East respiratory syndrome coronavirus (MERS-CoV)

Flaviviruses - Group B Arboviruses
--Japanese encephalitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)
--West Nile virus (WNV)
--Yellow fever virus
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Orthomyxoviruses
-- Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses
--Monkeypox virus

Prions
--Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, Footnotes and References of Sections I through IV, for containment instruction)

Retroviruses
--Human immunodeficiency virus (HIV) types 1 and 2
--Human T cell lymphotropic virus (HTLV) types 1 and 2
--Simian immunodeficiency virus (SIV)

Rhabdoviruses
--Vesicular stomatitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)

Risk Group 4 Agents

- **Risk Group 4 Bacterial Agents**
  - None

- **Risk Group 4 Fungal Agents**
  - None

- **Risk Group 4 Parasitic Agents**
  - None

- **Risk Group 4 Viral Agents**

Arenaviruses
--Guanarito virus
--Lassa virus

--Junin virus (except the candid #1 vaccine strain listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
--Machupo virus
--Sabia

Bunyaviruses (Nairovirus)
--Crimean-Congo hemorrhagic fever virus

Filoviruses
--Ebola virus
--Marburg virus

Flaviruses - Group B Arboviruses
--Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
--Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
--Equine Morbillivirus (Hendra virus)

Hemorrhagic fever agents and viruses as yet undefined

Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work. A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses
--Herpesvirus ateles
--Herpesvirus saimiri
--Marek's disease virus
--Murine cytomegalovirus

Papilloma viruses
--Bovine papilloma virus
--Shope papilloma virus

Polyoma viruses
--Polyoma virus
--Simian virus 40 (SV40)

Retroviruses
--Avian leukosis virus
--Avian sarcoma virus
--Bovine leukemia virus
--Feline leukemia virus
--Feline sarcoma virus
--Gibbon leukemia virus
--Mason-Pfizer monkey virus
--Mouse mammary tumor virus
--Murine leukemia virus

--Murine sarcoma virus

Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

3. BIOSAFETY LEVELS AND CONTAINMENT

Pathogens and infectious agents should be handled and contained depending upon their biological characteristics. These characteristics or factors provide for an indication of the associated risk of exposure and the risk of disease of the host. The factors which are associated with the risk of exposure are the host's

work activity, proficiency, age, sex, immune status and medications being used. The factors associated with the risk of disease to the pathogen include virulence, infectious dose, route of infection, toxigenicity, agent's host range, and the availability of effective preventive measures and treatment. Other factors which determine how susceptible one is to an infectious agent are the host's natural defense mechanisms and the chance for opportunistic infection.

The World Health Organization (WHO) has classified infective microorganisms by risk group. Refer to Appendix 1 for definition of the groups. However, it is standard to classify infectious agents by biosafety level classification rather than the WHO Risk Groups. Therefore, that classification system will be used and referred to in this manual.

The CDC/NIH biosafety levels are also used to describe and refer to laboratory types which are used to contain the infectious agents. These are described in the "Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. The levels are used to designate laboratories according to their design features, construction and containment facilities. Biosafety Levels 1 and 2 and labs are basic labs, Biosafety Level 3 lab is called a containment lab, and a Biosafety Level 4 lab is a maximum containment lab. These biosafety levels are described below.

Since the modes of transmission for varying infectious agents are so different, precautions must be taken to avoid exposure. Examples of indirect transmission are the foods we eat and the water we drink or contact with a carrier. Direct transmission is through human-to-human contact, air-borne transmission and needle sticks. *It has been shown that needle sticks are responsible for the greatest number of work related infections in health care workers.*

Depending on the type of biological system used in the specific research, lab facilities and equipment must be available to safely handle and manipulate the biological research materials.

3.1 Biosafety Level Definitions

Biosafety Level 1 (BSL-1)

Biosafety Level 1 is suitable for work involving agents of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Standard Microbiological Practices (BSL-1).

- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
- Work surfaces are decontaminated once a day and after any spill of viable material.
- All contaminated liquid or solid wastes are decontaminated before disposal.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- Persons wash their hands: (a) After they handle materials involving organisms containing recombinant DNA molecules and animals, and (b) before exiting the laboratory.
- All procedures are performed carefully to minimize the creation of aerosols.
- In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Special Practices (BSL-1).

- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- An insect and rodent control program is in effect.

Containment Equipment (BSL-1).

- Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Laboratory Facilities (BSL-1).

- The laboratory is designed so that it can be easily cleaned.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing.
- If the laboratory has windows that open, they are fitted with fly screens.

Biosafety Level 2 (BSL-2)

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1)

laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

Standard Microbiological Practices (BSL-2).

- Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All contaminated liquid or solid wastes are decontaminated before disposal.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- Persons wash their hands: (a) after handling infectious materials including organisms containing recombinant DNA molecules and animals, and (b) when exiting the laboratory.
- All procedures are performed carefully to minimize the creation of aerosols.
- Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Special Practices (BSL-2).

- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
- The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.
- When the infectious materials including organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.
- An insect and rodent control program is in effect.
- Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g.,

cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

- Animals not involved in the work being performed are not permitted in the laboratory.
- Special care is taken to avoid skin contamination with infectious materials, including organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.
- All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
- Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing infectious materials including organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.
- Spills and accidents which result in overt exposures to infectious materials including organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/ORDA. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.
- A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Containment Equipment (BSL- 2).
Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:

- Procedures with a high potential for creating aerosols are conducted.
- These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.
- High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Laboratory Facilities (BSL- 2).

- The laboratory is designed so that it can be easily cleaned.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing.
- If the laboratory has windows that open, they are fitted with fly screens.
- An autoclave for decontaminating laboratory wastes is available.

Biosafety Level 3 (BSL-3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BSL-3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BSL-2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BSL-3 are rigorously followed. The decision to implement this modification of BSL-3 recommendations should be made only by the Principal Investigator.

Standard Microbiological Practices (BSL-3).

- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All contaminated liquid or solid wastes are decontaminated before disposal.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.
- Persons wash their hands:
    - (i) after handling infectious materials including organisms containing recombinant DNA molecules, and handling animals, and
    - (ii) when exiting the laboratory.
- All procedures are performed carefully to minimize the creation of aerosols.

- Persons under 16 years of age shall not enter the laboratory.
- If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BSL-3 level physical containment, they shall be conducted in accordance with all BSL-3 level laboratory practices.

Special Practices (BSL-3)

- Laboratory doors are kept closed when experiments are in progress.
- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
- The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.
- When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.
- All activities involving infectious materials including organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.
- The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials including organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean up.
- An insect and rodent program is in effect.
- Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.
- Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
- Molded surgical masks or respirators are worn in rooms containing experimental animals.

- Animals and plants not related to the work being conducted are not permitted in the laboratory.
- Laboratory animals held in a BSL-3 area shall be housed in partial-containment caging systems, such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter
- bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors. *Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.*
- All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
- Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps. (See schematic in Appendix 2.)
- Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing infectious materials including organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
- Spills and accidents which result in overt or potential exposures to infectious materials including organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/ORDA. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
- Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.
- A project-specific biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.
- *Alternative Selection of Containment Equipment* (BSL-3)
  Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BSL-3 laboratory using containment equipment specified for the BSL-2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BSL-3 laboratory using containment equipment specified for the BSL-4 level of

physical containment. Alternative combination of containment safeguards may be considered. Refer to the CDC/NIH Guidelines for these safeguards.

Containment Equipment (BSL-3).
Biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with infectious materials including organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

Laboratory Facilities (BSL-3)

- The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.
- The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
- Windows in the laboratory are closed and sealed.
- Access doors to the laboratory or containment module are self-closing.
- An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.
- A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper.
- The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the

cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection that avoids any interference with the air balance of the cabinets or building exhaust system.

Biosafety Level 4 (BSL-4)

The description for BSL-4 will not be included here as such facility is currently unavailable at DU. If you are interested, please refer to the NIH Guidelines for a full description of a maximum containment facility.

Laboratory Facilities

Secondary Barriers - Laboratory Design

The laboratory must include specific design features which can only be put into place during the initial construction or remodel of a work area. Therefore, it is necessary to consider what the current as well as future needs might be of a given area. It is not possible to plan or anticipate all future needs, so it is necessary to provide minimally for a standard laboratory suite.

The standard or basic teaching laboratory is denoted as a biosafety level 1 (BSL 1) laboratory. The biosafety level 2 laboratory is often referred to as a modified basic laboratory, as it has the same design features as the BSL-1 laboratory with the added requirement of a biosafety cabinet as a standard feature.

The containment laboratory is the type of facility specified by the CDC/NIH Biosafety guidelines for Biosafety Level 3 infectious agents. It is a restricted lab with specific design features which must be accomplished during a construction phase. These features are identified earlier in this section.

The maximum containment facility is required for infectious agents and pathogens which are in the biosafety level 4. It is not anticipated that these pathogens would ever be handled at the DU campus. Currently there are only 10 such facilities in the U.S.

Primary Barrier - Equipment
The primary barriers are those which are enclosed by the secondary barrier, these primary barriers include equipment and instrumentation which would be used to contain biohazardous materials at the source. Examples of these are: biosafety cabinets, closed ventilated animal cages, safety centrifuge cups and safety blenders. Of these, the biological safety cabinet is the single most important containment device.

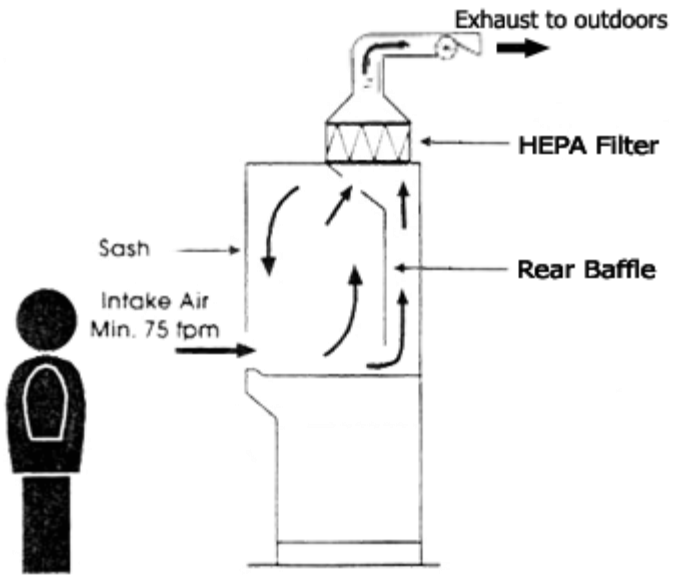
3.2 Biosafety Cabinets

Biological safety cabinets are classified as Class I, Class II, or Class III cabinets. The most common type biosafety cabinets used at DU are Class II, Type A and Type B2. Biosafety cabinets should not be confused with clean air benches or glove boxes. Clean air benches offer no protection to the environment or personnel. Room air is pulled into the cabinet by a fan motor. The air then passes through a HEPA or standard air filter before the air passes over the product. Glove boxes (class III) are a totally enclosed, ventilated cabinet of gas tight construction, which is maintained under negative pressure and provides for worker protection. Operations are performed through attached rubber gloves. Other terms often used are laminar flow benches and tissue culture hoods. For consistency, this manual will refer to biosafety cabinets and types as described below.

Classification	**Biosafety Level**	**Application**
Class I	1, 2, 3	low to moderate risk biological agents
Class II	1, 2, 3	low to moderate risk biological agents
Class III	4	high risk biological agents

The common Biosafety Cabinet features are as follows:

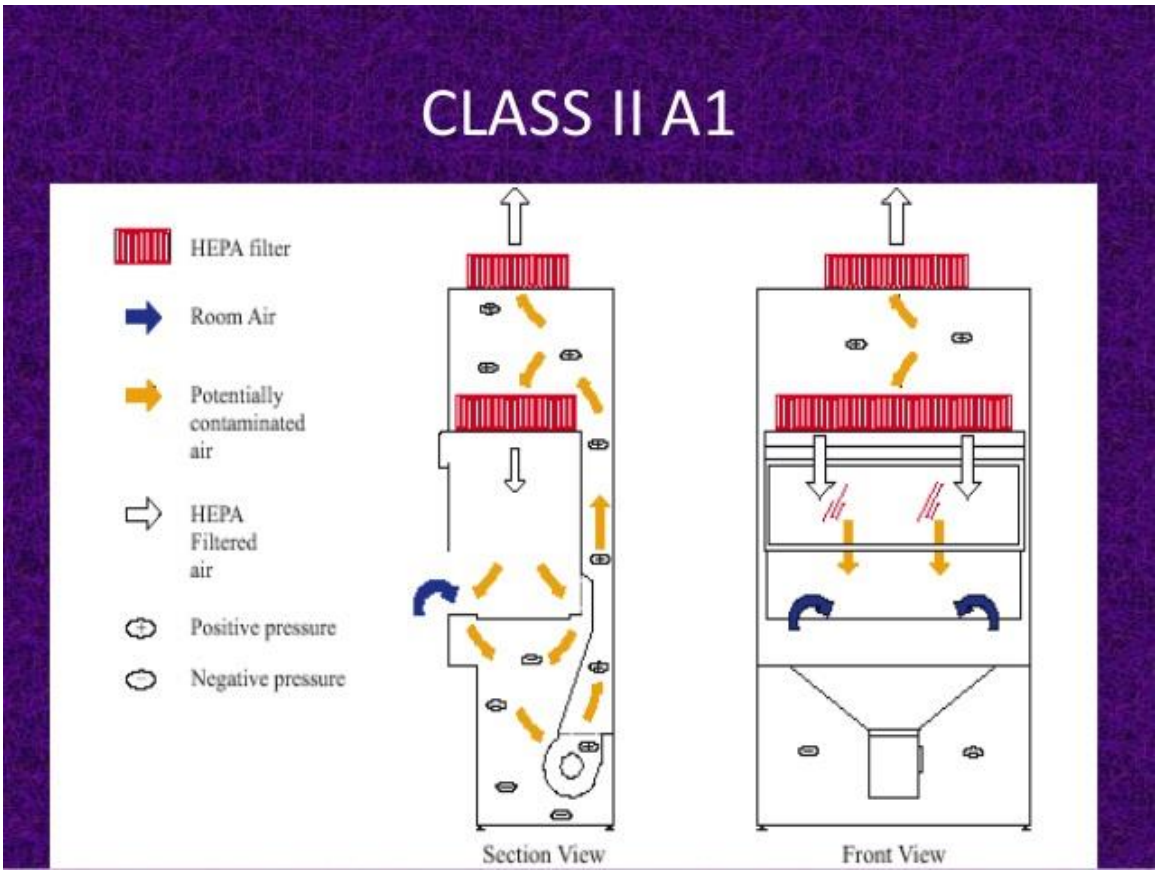
- Class I - **Class I cabinets** provide personnel and environmental protection but no product protection. In fact, the inward flow of air can contribute to contamination of samples. This class of cabinet does not provide protection for the product. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. Inward airflow is maintained at a minimum velocity of 75 ft./min (0.38 m/s). These BSCs are commonly used to enclose specific equipment (e.g. centrifuges) or procedures (e.g. aerating cultures) that potentially generate aerosols. BSCs of this class are either ducted (connected to the building exhaust system) or unducted (recirculating filtered exhaust back into the laboratory).



- Class II, there are four types: Type A1 (formerly A), Type A2 (formerly A/B3), Type B1, and Type B2. Each type's requirements are defined by NSF International Standard 49 which in 2002 reclassified A/B3 cabinets (classified under the latter type if connected to an exhaust duct) as Type A2. About 95% of all biosafety cabinets installed are Type A2 cabinets. (Actually, now there is another option “Class 2 C1” offered by one manufacturer, but not described here).

- A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward airflow for personnel protection, and HEPA filtered mass recirculated airflow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation. Class II biosafety cabinets are subdivided into three types.

  The **Class II Type A1 cabinet**, formerly known as Type A, has a minimum inflow velocity of 75 ft./min. The filtered makeup air is divided equally over the work surface at about two to six inches above the work surface. Exhaust is drawn at the bottom of the cabinet where it rises to the top. At the top of the cabinet, 70% of the air recirculates through the supply HEPA filter, the other 30% of air exhausted through the exhaust HEPA filter. This is due to the relative sizes of the two filters, and dampers typically allow the adjustment of this ratio. This type is not safe for work with hazardous chemicals except when ducted, usually with a "thimble" or canopy hood to avoid disturbing internal air flow.



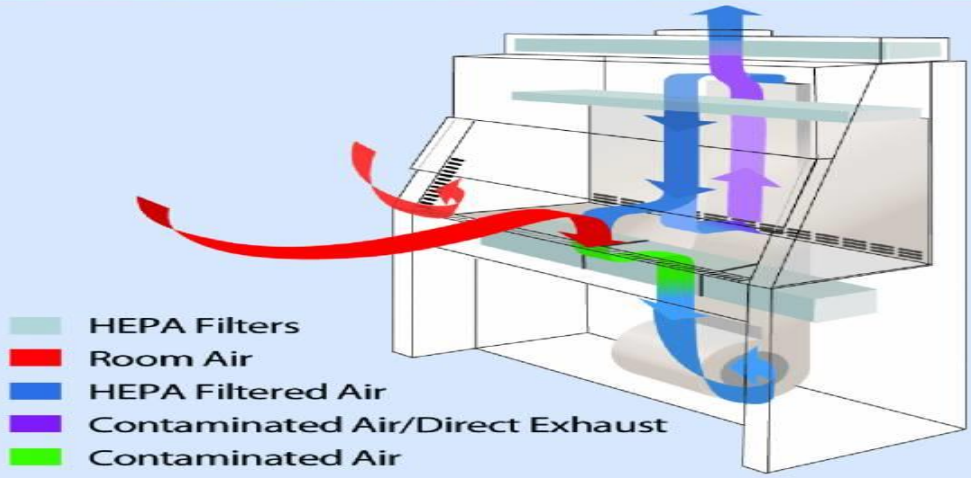
Class 2 Type A1

The **Class II Type A2 cabinet**, formerly designated A/B3, has a minimum inflow velocity of 100 ft./min. A negative air pressure plenum surrounds all contaminated plenums that are under positive pressure. In other respects, the specifications are identical to those of a Type A1 cabinet

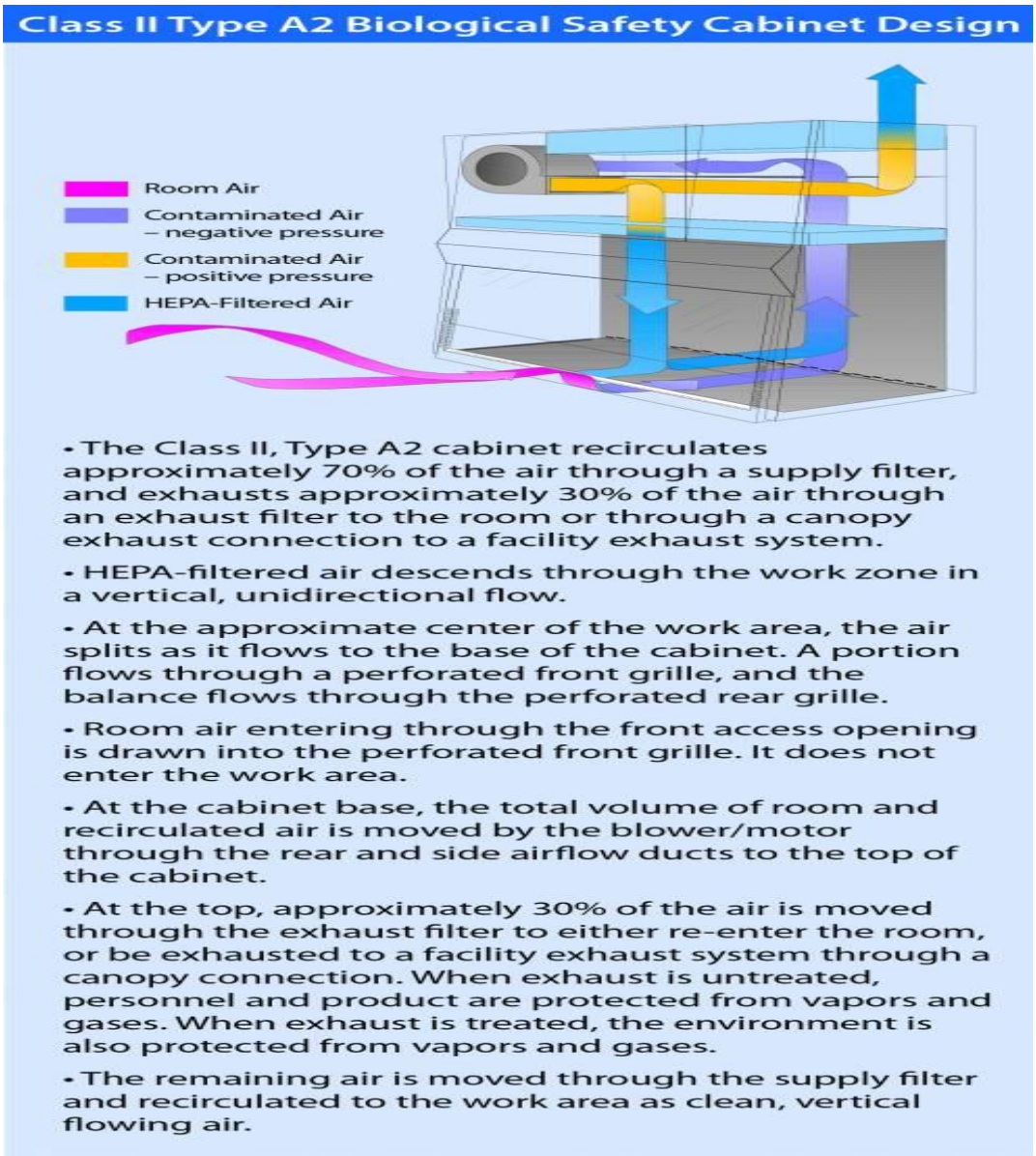
Class II, Type A2 Airflow

- The Type B1 and B2 cabinets have a minimum inflow velocity of 100 ft./min, and these cabinets must be hard-ducted to an exhaust system rather than exhausted through a thimble connection. In contrast to the type A1 and A2 cabinets, B1 cabinets split the airflow so 60% of air is exhausted and only 40% is recirculated, with the air collected through the rear grille being exhausted, and air through the front grille being recirculated. Since exhaust air is drawn from the rear grille, the CDC advises that work with chemicals be conducted in the rear of the cabinet. The Type B2 cabinet is expensive to operate because no air is recirculated within. Therefore, this type is mainly found in such applications as toxicology laboratories, where the ability to safely use hazardous chemicals is important. Additionally, there is the risk that contaminated air would flow into the laboratory if the exhaust system for a Type B1 or B2 cabinet were to fail. To mitigate this risk, cabinets of these types generally monitor the exhaust flow, shutting off the supply blower and sounding an alarm if the exhaust flow is insufficient.

Class II Type B1 Biological Safety Cabinet Design



- From above the work surface, HEPA-filtered air descends in a vertical unidirectional airflow over the work area. This air splits at the approximate center as it flows to the base of the cabinet.
- Approximately 60% of descending air is pulled directly through the rear grille of the work area into a dedicated negative pressure plenum. This air passes through an exhaust HEPA filter, then to an appropriate treatment system or outdoors via the facility's exhaust system.
- Approximately 40% of the descending air is pulled forward where it mixes with room air entering the perforated front grille. This air passes through a HEPA supply filter directly below the work surface, then is circulated under positive pressure through a duct to the top of the cabinet, then through another HEPA supply filter, where the process is repeated.



- A **Class III cabinet** is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.
  National Sanitation Foundation Standard 49. 2002.

The previous information is provided to introduce the user to the types of cabinets available and to inform users that not every biosafety cabinet is the same. If you have any questions about the appropriateness of a biosafety cabinet for the type of research being conducted, please contact the ES&H Office at ext. 1-7501 to assist in determining the level of protection required. The manufacturer's technical representatives are also able to provide the investigator with pertinent information.

Startup Work Practices with a BSC

All laboratory personnel should be familiar with biosafety cabinets. There are numerous types and nomenclature depending upon their usage. The proper biosafety cabinet should be used for the appropriate manipulations of biological materials. If you would like information regarding the usage, specifications or in-service training of biosafety cabinets, please contact the DU EH&S Department at 1-7501.

Startup procedures for the biosafety cabinet (BSC):

- Turn off the ultraviolet (UV) lamp (if one is used).

- Turn on fluorescent light, inspect air intake grilles for obstructions and foreign materials and remove any obstructions.
- Adjust view screen to proper height.
- Turn on blower and allow five minutes to purge the air.
- Wash hands and arms with mild soup, put on a rear-fastening, long-sleeved gown with tight fitting cuffs. Put on a pair (or two) of high quality latex or nitrile gloves.
- Disinfect the interior surfaces of the BSC by wiping down with an appropriate disinfectant and place a plastic-backed pad on the work surface without covering the air intake/exhaust grilles.
- Put all items for the experiment in the BSC and keep clean items segregated from dirty items.
- Organize the material so that dirty "contaminated" items are not passed over (cross-contaminate) clean items. Allow air to stabilize for a few minutes before starting work.
- Work from "clean" to "dirty" areas. Work at least six inches back from the front air intake grille. (See schematic in Appendix 3.)

Containment Centrifuges A containment centrifuge is one which is equipped with a containment feature which protects the laboratory atmosphere from the release of potentially infectious aerosolized materials. Aerosolization of the product in a centrifuge can occur when a bottle or tube leaks or ruptures. A containment device can be a secondary gasket to seal the rotor or centrifuge lid or safety cups and canisters which would contain a ruptured tube and/or specimen. For more detailed information, contact your centrifuge manufacturer.

4. LABORATORY PRACTICES AND TECHNIQUES

The following practices and techniques are those which differentiate BSL-1 labs from BSL-2 labs. BSL-2 labs require that access be controlled, standard microbiological practices and universal precautions be followed, including the use of appropriate personal protective equipment (PPE).

Access Control

Access to the research laboratory should be limited or restricted to authorized personnel only. The laboratory director or supervisor shall be responsible for the laboratory and authorizing access. When experiments are in progress where access restrictions are appropriate, the doors shall be closed and a warning sign posted.

The standard microbiological practices which are referred to in the CDC and NIH guidelines include hygienic and operational practices which are critical in providing for a safe work environment and assuring a viable research product is produced. These practices are also necessary for minimizing and/or eliminating the risk of occupational exposure to infectious and potentially infectious substances.

Hygienic Practices

- No eating, drinking, manipulating contact lenses, applying cosmetics or lip balm in the laboratory.
- Wash hands often. Wash for at least ten seconds with mild soap and water after handling potentially contaminated materials or equipment, after removing gloves, and before leaving the laboratory.
- Wear protective gloves when handling potentially pathogenic, oncogenic, or contaminated materials or equipment. Remove gloves when you leave the lab.
- Keep hands away from mouth, nose, eyes, and face.
- Protective laboratory coats, gowns, or uniforms are advised when handling research materials.
- Protective clothing and/or devices shall not be worn outside of the laboratory, (and especially not to eating areas).
- Protective clothing and/or devices contaminated by potentially pathogenic materials should be decontaminated before laundering, disposal or repair.

Operational Practices

- Mechanical pipetting devices or pipetting aids must be used.
- Mouth pipetting is prohibited.
- Select a pipetting device for biohazardous material that can be easily decontaminated or is disposable.

- Use a pipette tip with filter where appropriate to protect the pipetting device from contamination.
- All procedures are performed carefully to minimize the creation of aerosols.
- Place a plastic-backed, absorbent paper pad on the work surface.
- Contaminated pipettes should be discarded horizontally into a pan containing enough disinfectant to give complete immersion or placed directly into an infectious waste box with red bag liner.
- Avoid use of syringes and needles. Substitute blunt needles or cannulas when possible.
- Vacuum lines must be protected from potential contamination by the use of a disposable hydrophobic filter. (Contact HSD for information on filters.)

Decontamination

Cleaning and decontamination of any lab or work area designated with a red Biohazard sign on the door indicating use of BSL-3 agents is the responsibility of laboratory personnel. All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood of becoming contaminated with blood or other potentially infectious materials shall be inspected and decontaminated on a regularly scheduled basis and cleaned with fresh 1:10 bleach solution immediately and also when visible contamination is identified (see section 7, Decontamination and Disinfection procedures).

Control of Aerosols

To control the aerosolization of infectious materials use containment equipment such as capped safety centrifuge cups, sealed centrifuge heads, safety blenders, biosafety cabinets and vacuum traps (see figure in Appendix 2).

- Use filter tops on animal cages.
- Use a biological safety cabinet for work with infectious agents or materials or tissue cell cultures suspected of containing pathogenic and/or carcinogenic agents.

Housekeeping Practices

General housekeeping of the laboratory work area is the responsibility of the laboratory personnel. The laboratory director must assure that the work area is maintained in a clean and orderly fashion.

- Properly store equipment and supplies to reduce clutter.
- At the end of the workday, all infectious or hazardous materials should be "secured".
- At the end of the work shift or day all work surfaces must be decontaminated. Remove the absorbent bench cover and dispose of into red bag. Wipe down workbench with a 10% bleach/water solution.

- Avoid dry mopping or sweeping. Wet mop will be performed by Central Environ- mental Services on a regularly scheduled basis.
- Cleaning equipment and supplies used to clean the laboratory should be decontaminated before reuse or disposal.
- Animal handling shall be performed with proper precautions.

Emergency Plan

Each laboratory should have the pre-arranged emergency procedures specifying the appropriate response to potential emergencies. Accidents and spills of infectious materials will be discussed in Emergency procedures below.

Procedures to Secure a Lab Before Leaving

Research laboratories require that they be secured when there are no personnel in the work area. This is mandated by the Colorado Hazardous Waste Regulations and Colorado Radiation Control Regulations which pertain to DU laboratories when hazardous or radioactive materials or wastes are stored there. The following steps should be taken in securing DU research laboratories.

- Properly store materials: biological, chemical and radioactive
- Remove all items from the biological safety cabinet.
- Decontaminate material and work surfaces.
- Turn off equipment, flames, steam supply, electrical appliances.
- Perform routine housekeeping.
- Disinfect reusable equipment.
- Remove protective gloves.
- Wash hands.
- Remove other protective equipment and wash hands.
- Turn off lights and secure laboratory door.

Emergency Procedures

A. Large Biohazard spill outside of BSC
    1. Block aerosol transmission. Hold breath - leave room - close and post door. Call extension 1-3000 and/or 1-7501.
    2. Remove contaminated clothing and wash.
    3. Do not leave area until emergency responders have arrived.
    4. Use an appropriate disinfectant with a 20-minute contact time followed by mop up.
    5. Remove PPE and wash hands.

B. Minor Biohazard Spill Outside of Biosafety Cabinet
    1. Hold breath - leave area - post warning for others.
    2. Remove contaminated clothing and wash.
    3. Wear clean PPE (double glove, lab coat, glasses or goggles and mask if splash likely) and contain spill with absorbent.

4. Overspray the absorbent and biohazardous materials with an appropriate disinfectant.
5. Allow 10-20 minute contact time followed by mop-up.
6. Dispose of spill and mop-up materials in appropriate biohazard waste bag/tub.
7. Remove PPE and wash hands thoroughly.

5. PERSONAL PROTECTIVE EQUIPMENT

All DU research staff shall use personnel protective equipment (PPE) when necessary to prevent exposure to infectious substances. The type of protective equipment must be appropriate for the procedure being performed and the type of exposure anticipated. Personnel shall don the appropriate PPE before beginning procedures when the possibility of being exposed to potentially infectious substances exists.

Gloves

1. Disposable non-sterile latex, (due to latex allergies, latex gloves are now banned in healthcare settings, and are becoming much less common in lab settings), non-latex, nitrile, or vinyl gloves shall be worn for situations listed below.
2. Sterile gloves shall be worn only for procedures normally requiring sterile control and aseptic technique.
3. Gloves shall be examined for visible defects before donning, after donning, and before commencing work. *Note: A good way to check gloves for defects if to blow air into them.*
4. Gloves with any visible defects shall be discarded, followed by immediate handwashing.
5. Gloves shall be changed when grossly soiled.
6. All gloves shall be removed before leaving work area or before touching "clean items" that must remain clean.
7. Disposable gloves are never to be washed or reused.
8. All gloves potentially contaminated with infectious substances shall be discarded into red biohazard bags.

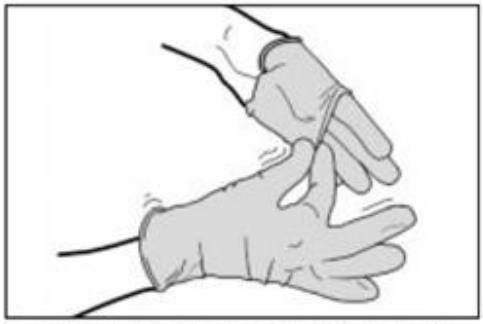
The appropriate type of gloves shall be worn in situations including, but not limited to, the following:

- For procedures involving contact with blood or other bodily fluid/substances,
- When touching patient's non-intact skin, mucous membranes, and skin rashes,
- When handling or touching items or surfaces (including specimen containers, instruments, countertops, etc.) contaminated with blood or other potentially infectious materials,
- When a DU worker has cuts, scratches, dermatitis, or other breaks in the skin,
- When handling biohazard bags and their secondary containers,
- When handling/touching any other potentially infectious substances,
- When the potential for contact with potentially contaminated toxic material exists,
- Spill cleanup or disposal of biohazards and chemicals, including biohazard bagged material, and
- When handling radioactive material.

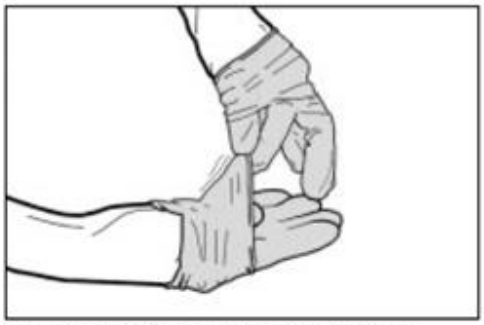
- Double gloving is recommended in situations when gross contamination of gloves with infectious materials is likely. Two pairs may be worn in higher risk areas such as when cleaning up spills. In this case one pair is used for general laboratory work and the second pair is worn for work in the Biosafety cabinet. *Double gloving is also recommended when handling locally anaesthetized animals infected with infectious agents posing substantial risk to humans (e.g. Hepatitis B virus, HIV).*
- Utility or nitrile gloves are best for cleaning, maintenance engineering and other activities which may easily rip latex or vinyl gloves.
- Intact utility gloves can be reused after decontamination by washing in disinfectant-detergent. These gloves are best for housekeeping and facilities departments.
- Nitrile gloves are recommended when work is performed in an environment when hazardous chemicals are used in a system with biohazards. These offer greater chemical resistance and fewer holes than latex or vinyl. Please note that nitrile is not appropriate for all hazardous chemicals.
- Leather and/or thick, cloth gloves maybe used for some animal handling and waste handling procedures. Leather should not be worn when potential exists for contamination with chemicals or infectious agents.

Removal of Gloves Technique

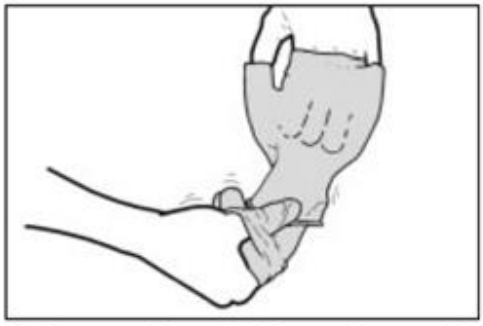
1. Use the following pictures as a guide to help you remove gloves safely
2. Avoid touching the outside of the gloves. Only touch the inside
3. Wash hands after removing and disposing of gloves in a sealable bag



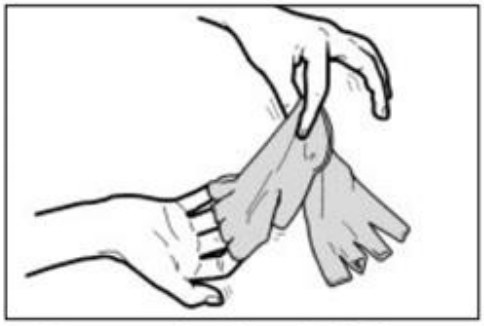
1. Grasp one glove at wrist and pull down to knuckles.



2. Grasp other glove at wrist and pull down to knuckles.



3. Grasp wrist end of one glove and pull it off completely.



4. Remove other glove in similar way touching only the inside of gloves.

5. Dispose of gloves in an appropriate container.

6. Wash hands after removing and disposing of gloves.

Eye Protection

All DU laboratory personnel are required to wear eye protection when the risk of eye exposure to hazardous/infectious/radioactive materials exists. Depending on the nature of the hazard, this would be at a minimum, safety glasses, and may include goggles and face shields.

Examples of such situations include:

- A potential for splash of blood, body fluids or chemicals.
- Cleaning up a spill of potentially infectious materials, including tissue culture stocks, blood samples, any Class 2 or higher agents, or rDNA products from Class 2 or higher agents,
- Opening a sonicator, blender, or homogenizer following agitation of a potentially infectious material, i.e. human tissues or organs,
- Opening a package of potentially infectious materials packed at lower elevations,
- Wiping down work surfaces with disinfectant at the end of the work shift,
- Pipetting or dispensing potentially infectious solutions, and
- Handling or manipulating any infectious materials where there is a high risk of splatter, spray or aerosolization of the materials.

Face (nose and mouth) protection

Appropriate face protection should be worn when a potential for sprays, mists, splashes or aerosols exists. The types of face protection available include surgical masks, molded particulate masks, respirators, dust masks, and face shields.

In general face protection should be used as follows:

- Surgical masks or face shields shall be worn during invasive procedures when there is a potential for spray or splashes,
- Surgical masks or face shields shall be worn when cleaning up a spill of potentially infectious materials,
- Molded surgical masks or respirators are worn in Biosafety Level 3 rooms containing experimental animals, and
- Approved N-95 respirators are worn when there is a risk of exposure to an airborne infectious agent, i.e. M. tuberculosis.

Note: When there is a potential for aerosolization of infectious materials, a face shield should not be used instead of approved respirators. Please note that the usage of a respirator requires a medical exam, fit testing and formal training, 29 CFR 1910.134. For more information, call EHS at 1-7501.

Gowns

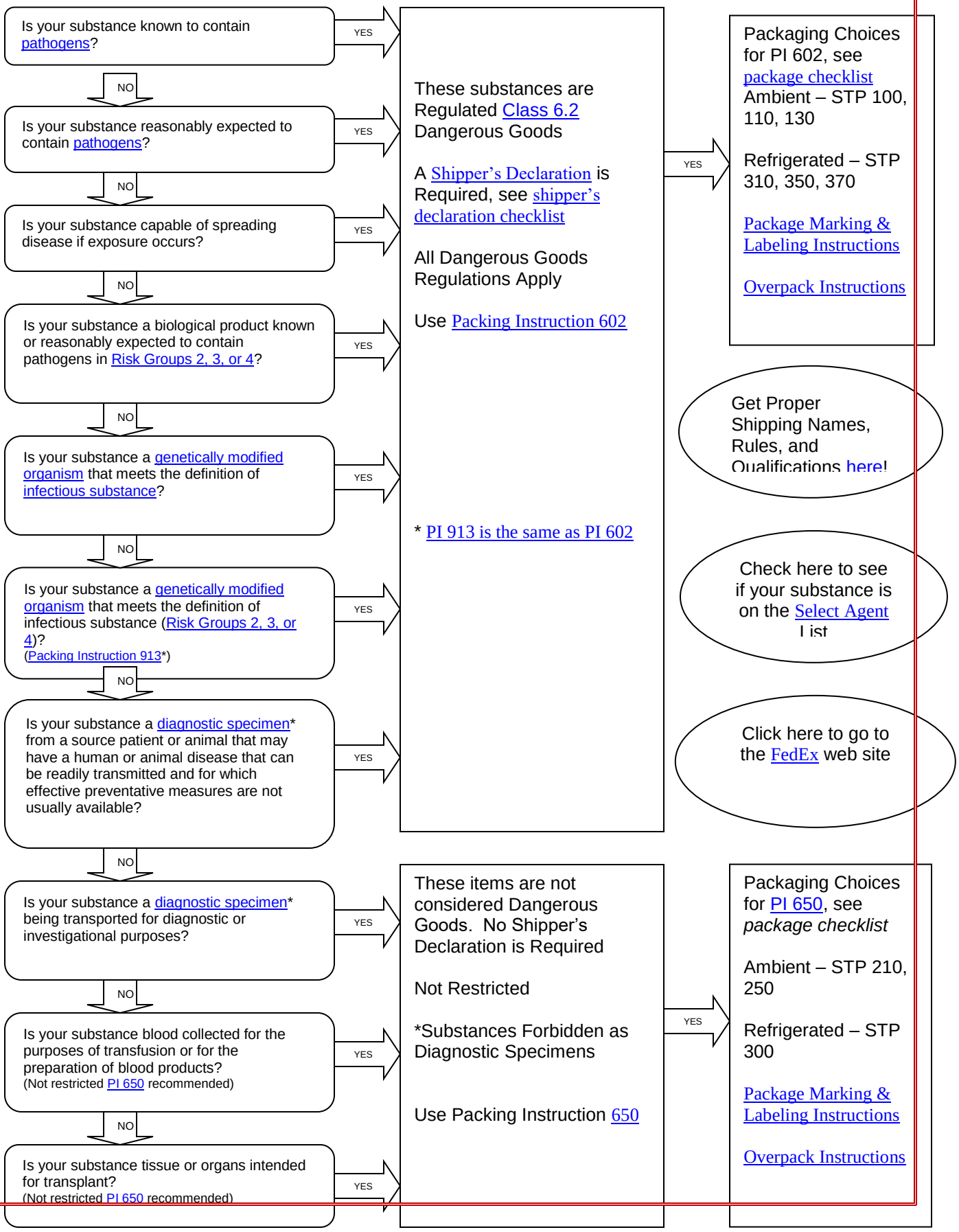
Gowns with solid front or wrap-around with knit cuffs are recommended for people working in biological safety cabinets and Biosafety Level 3 Laboratories.

Impervious laboratory coats/cover gowns shall be worn for all procedures that:

- have the potential for generating soiling, splashing, or spraying of clothes/scrub attire with infectious agents, blood, body fluids, or other potentially infectious substances
- have the potential for soiling, splashing, or spraying of clothes with toxic materials (e.g. hazardous chemicals and/or reagents, radioactive materials)
- involve performing spill cleanup or disposal of biohazardous material and chemicals.

Impervious gowns/laboratory coats/ aprons shall be removed before leaving the work area and must be decontaminated prior to laundering. Decontaminate the garment by chemical disinfection or autoclaving, as is appropriate.

6. Packaging/Shipping Infectious Materials and Specimens



7. Select Agents

Select Agents and Toxins List

The following biological agents and toxins have been 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. Here is a list of excluded agents and toxins.

HHS and USDA Select Agents and Toxins
7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS
Abrin
Bacillus cereus Biovar *anthracis**
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins
containing the following amino acid sequence
$X_1CCX_2PACGX_3X_4X_5X_6CX_7$)[1]
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus[3]
Ebola virus*
*Francisella tularensis**
Lassa fever virus
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

OVERLAP SELECT AGENTS AND TOXINS
*Bacillus anthracis**
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus[3]

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)

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)*
*Yersinia pestis**

Phoma glycinicola (formerly Pyrenochaeta glycines)
Ralstonia solanacearum
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. 9/10/13

***** VIOLATIONS**

As of January 6, 2017, the Department of Health and Human Services (HHS) Office of Inspector General (OIG) places into effect Civil Monetary Penalty (CMP) rules added to 42 CFR Part 1003: Subpart I – CMPs for Select Agent Program Violations: §1003.900 Basis for civil money penalties, §1003.910 Amount of penalties, and §1003.920 Determinations regarding the amount of penalties.

The HHS OIG will now consider the following factors in all 42 C.F.R. Part 73 Select Agent Program violations:

§1003.920 Determinations regarding the amount of penalties.

In considering the factors listed in §1003.140, aggravating circumstances include:

(a) The Responsible Official participated in or knew, or should have known, of the violation;

(b) The violation was a contributing factor to an unauthorized individual's access to or possession of a select agent or toxin, an individual's exposure to a select agent or toxin, or the unauthorized removal of a select agent or toxin from the person's physical location as identified on the person's certificate of registration; or

(c) The person previously received an observation, finding, or other statement of deficiency from the Department or the Department of Agriculture for the same or substantially similar conduct.

The TRR and LA committees determined to share this information with the FSAP community as §1003.920 elicited concerns that the vague language and potentially broad interpretation of this regulation may lead to inconsistent and steeper penalties. To see the entire changes, please see the regulations at https://www.gpo.gov/fdsys/pkg/FR-2016-12-07/pdf/2016-28293.pdf#page=21 or http://www.ecfr.gov/cgi-bin/text-idx?SID=d71e546e10776faf4dce1550c467b1c2&mc=true&node=sp42.5.1003.i&rgn=div6 .

8. DECONTAMINATION AND DISINFECTION

(Types of Treatment for Infectious Materials)

Definitions

Whether preparing infectious materials for disposal or cleaning up a spill, it is important that the materials be treated properly to assure disinfection and/or decontamination.

Disinfection
The elimination of most or all pathogenic microorganisms on inanimate objects (with the exception of bacterial spores.) A disinfectant generally destroys a specific target organism. This is usually accomplished by use of liquid chemicals or wet pasteurization.
Sterilization
The complete elimination or destruction of all forms of microbial life. It is accomplished in the hospital or research lab by either physical or chemical processes. Steam sterilization (autoclaving), dry heat, ethylene oxide gas (gas sterilization), and liquid chemicals are common methods.
Decontamination
The destruction of microorganisms to some lower level, but not necessarily to zero. It is accomplished by sterilization or dis- infection of the materials containing the pathogenic micro- organisms.

If a material is -cidal, it kills or inactivates an agent (i.e. bacteri-, viru-, fungi-, tuberculo-, microbi-, spori-, or germi-.) Many disinfectants will list its "cidal" activity. To determine whether a specific agent will be killed, the label must be read thoroughly. Usually a manufacturer will include a listing of agents for which industry standard testing has been conducted.

8.1 Chemical disinfection

Chemical disinfection is accomplished by dosing an infectious material with an appropriate amount of disinfectant. An appropriate disinfectant is one which will kill or reduce the numbers of the targeted agent to an acceptable level. The common classes of chemical disinfectants are alcohols, chlorine compounds, phenolic compounds, quaternary ammonium compounds, and iodophors. Manufacturers of the types of disinfectants combine detergents with these materials to improve cleaning capabilities of their products. The manufacturer's comments must always be read carefully to assure appropriateness for a particular agent or agents.

Infectious Liquids

Infectious liquids have historically been chemically disinfected or steam sterilized. These treated liquids were then appropriate for sewer disposal. Whenever using any treatment method to render the materials disinfected or sterilized, the appropriate method must be standardized. Many disinfected infectious liquid wastes can be safely disposed into the sanitary sewer if the proper steps are followed. Liquid infectious wastes must be chemically treated with bleach or autoclaved (steam sterilized) prior to disposal into any drain. Mixed wastes (chemical and radioactive wastes with infectious wastes) are unsuitable for disposal into the sanitary sewer.

Disinfectants

Particular care should be taken when handling concentrated stock solutions of disinfectants. A majority of disinfectants are toxic to the human body by skin contact or inhalation. Personnel assigned the task of making up use - concentrations from stock solutions must be properly informed as to the potential hazards and trained in the safe procedures to follow. The concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Even a small droplet splashed into the eyes may cause blindness. Protective eye protection and long-sleeved garments and chemically resistant gloves, aprons, and boots should be worn to protect the skin from the corrosive and toxic effects of the disinfectant.

Special Considerations

The effectiveness of a disinfectant to kill or deactivate infectious agents will depend upon many factors. The following factors must be considered before assuming a disinfectant will be suitable for the particular application:

Type of Microorganism

Chemicals are not equally effective against the different types of microorganisms

Degree of Contamination

The degree of contamination affects the time required for disinfection, the amount of chemical required, and other variables. For example, the greater the degree of contamination, the longer the contact time needed for effective treatment.

Protein Content

Protein containing material (blood, plasma, feces, tissue, etc.) absorbs and inactivates some chemical disinfectants. Halogens, i.e., chlorine, combine readily with proteins. Therefore, when protein-containing materials are present in the waste, sufficient quantities of chlorine bleach must be added to provide the excess needed to react with the microorganism.

Type of Chemical

Different chemicals have different modes of action and levels of activity. It is important to understand the mode of action in order to select the appropriate chemical. For example, household bleach is ineffective as a disinfectant in either

acidic or basic conditions because the hypochlorous acid is no longer available to penetrate the cell wall.

Chemical Concentration/Quantity

Most chemicals have a range of concentrations that are suitable for use for disinfection. In the development of standard operating procedures, it is important to choose the proper concentration and quantity of chemical that are best used for the disinfection of each standard waste load.

Contact Time

It is essential that contact time be sufficient to allow for action of the chemicals on the microorganisms. The amount of contact time required for disinfection is proportional to the degree of contamination.

Other Considerations

Other factors that should be considered in establishing standard operating procedures for chemical disinfection include temperature, pH, mixing requirements, and aggregations of microorganisms.

Commonly Used Disinfectants

Disinfectant Group	Examples	Activity	Efficacy
Aldehydes	Formaldehyde, Paraformaldehyde, Gluteraldehyde (Cidex cold surface sterilization)	Biocidal activity Alkylation of carboxyl, hydroxyl and sulfhydryl group on proteins.	Surface and space decon as a gas and liquid
Halogen-based Biocides	Iodine, Chlorine, Wescodyne, Betadyne, Povidone-iodine and other iodophors are commercially available iodine-based disinfectants. Clidox® & Virex are chlorine based disinfectants.	Biocidal activity Bind to protein and modify sulfhydryl, amino, indole and phenolic groups. Acts as oxidizing agent.	Free organic matter, protein, will compete for chlorine ion reducing biocidal activity and making the disinfectant organic load dependent.
Quaternary Ammonium Compounds	Zephirin, CDQ, A-3	Cationic detergents, most effective against gram positive bacteria. Biostatic Generally ineffective against viruses, spores and Mycobacterium tuberculosis	Activity is reduced in presence of heavy organic matter loads. Good for water baths, incubators, and applications where

		Action is causing membrane damage and leakage, followed by denaturation.	halide or phenolic residues are not desired.
Phenolics	O-phenophenoate-base Compounds, Amphyl, O-syl, Tergisyl, Lysol, Vesphene, L-Phase and Expose	Biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria.	Biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria. Biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria.
Acids/Alkalis	In general acids are better than alkalis.	Increase of H and OH species in solutions which interfere with certain microbial functions. Total effect is not only dependent on pH alone. Weak organic acids are more potent than inorganic acids.	Disruption of 2 and 3 conformation of enzymes and structural proteins.
Heavy Metals	Silver nitrate and mercuric chloride used in 1:1000 aqueous solutions	Attack on protein sulfhydryl groups and disruption of enzyme functions.	Organic matter can reverse the disinfectant properties of mercurials.
Alcohols	Ethanol and isopropanol in 70 to 80% aqueous solutions	Disruption of cellular membranes, stabilization of lipids and denaturation of proteins by acting directly on S-H functional groups. Active against lipid-containing viruses, broad spectrum of bacteria, ineffective against spore-formers. Evaporate quickly, no residue, but evaporation interferes with contact time. Immersion is best.	Absolute alcohol is not as effective. Water is required for disinfection process

Sewer disposal of properly treated infectious liquids:

- Wear disposable gloves, eye protection, and a laboratory coat or gown.
- Do not pour infectious liquid down drains or sinks where people wash their hands. Pour the liquid close to the surface of the water to prevent the generation of droplets and aerosols.
- When the last of the properly treated infectious liquid is poured into the waste basin carefully rinse the remaining fluid down the drain with a cup of tap water. All of the infectious liquid must be flushed down the drain before turning on the water faucet. This procedure should minimize the formation of infectious aerosols. Rinse waste down the drain with plenty of running water.
- Disinfect the container if it is to be reused. Discard non-reusable containers into a red bag intended for infectious waste.

Types of fluids acceptable for sewer disposal:

- Urine, feces, vomit, and wastes associated with bedpans.
- Any bleach, detergent, or water that was used in cleaning operations.

8.2 Steam Sterilization

Steam sterilization is recommended for various types of infectious liquids. Some examples are: blood, serum, plasma, cultures and stocks of etiologic agents, fermentation wastes, and other infectious liquids not associated with radioactive or chemical materials. Steam sterilization is effective because the moisture available in the load sterilizes the material. The sterilization process, heating under pressure, causes the liquid materials to bubble or boil and may cause the bottles to break or explode if overfilled or improperly contained. This is sometimes referred to as a "hot-bottle explosion". For this reason, when autoclaving liquids use only vented closures - do not tightly seal bottles. Use glass bottles intended for autoclaving such as Type I borosilicate glass. Ordinary glass bottles are not designed for sterilization. Never autoclave flammable or other hazardous chemicals. Always carefully remove hot bottles from the autoclave and do not allow the bottles to be jolted. Do not move a bottle if boiling or bubbling is present. The bottles should be allowed to cool to the touch before attempting to move them from the sterilizer shelf or tray(s). For more detailed information on proper autoclaving techniques and procedures refer to the manufacturer's operations manual for your autoclave.

Autoclave Guidelines
DO NOT AUTOCLAVE items contaminated with solvents, volatile or corrosive chemicals, or items containing carcinogens, mutagens or teratogens. Contact DU EH&S (303-871-7501) for more information on how to dispose of non-autoclavable items properly.

Various items can be used to indicate proper function of an autoclave, ranging from the least reliant (autoclave tape) to chemical and biological Indicators

- Autoclave tape gives you a visual indication that the item has passed thru steam sterilization. DO NOT use as the only indicator of sterilization and decontamination!

- Chemical Integrator Strips consists of a steam and temperature sensitive chemical pellet, enclosed in a paper/foil envelope, which melts and migrates when exposed to autoclave conditions. The distance of migration depends on the exposure to steam, time, and temperature. This should be used with each autoclave run.

- Biological Indicators (BIs) provide the best assurance of sterility by challenging the sterilizer with quantifiable and highly heat resistant bacterial (B. stearothermophilus) spores. BIs must be incubated from 48-72 hours to obtain results, usually defined by a color change. These should be used monthly to assure proper function of the autoclave. Results should be recorded in a Quality Control log.

BEFORE Autoclaving

- Review the operator's manual for instructions as different makes and models of autoclaves have different controls.
- Wear appropriate PPE while loading and unloading the autoclave, including heat resistant gloves, lab coat, and eye protection. A face shield should be worn if a splash hazard is present. Use autoclavable polypropylene / polyethylene biohazard bags ONLY.

- Use a heat resistant secondary container to retain any leakage that may occur.

- DO NOT overfill bags or autoclave chamber as this decreases its effectiveness.

Autoclavable Infectious Waste Bags (may also be clear with biohazard symbol)

Biohazard bags placed in autoclavable secondary containers prior to autoclaving

DURING Autoclaving

- Use appropriate cycle times for the items you will be autoclaving:
  Sterilizing Clean Materials: 30 min. at 121°C and 15 psi
  Decontaminating Waste: 60 min. at 121°C and 15 psi
  Dense Loads: lengthen running time
  Liquids: use slow exhaust
  Glassware: use fast exhaust
- Segregate autoclave loads (infectious waste, liquid, or labware).
- DO NOT leave autoclaved material in autoclave overnight!

AFTER Autoclaving

- IT WILL BE HOT! - Allow materials to cool down for 15-20 minutes prior to their removal.
- ALWAYS make sure the pressure has gone to ZERO before opening the door!
- Use extreme caution when opening an autoclave door – there still may be steam inside the chamber after the pressure has dropped to zero which can cause severe burns.

9. BIOHAZARD/INFECTIOUS WASTE DISPOSAL

Definitions

Infectious waste is defined as waste, which is capable of transmitting disease. The most practical approach to defining infectious waste is to identify those categories of waste that have the greatest potential for transmitting disease. The following categories of waste are designated as infectious:

- **Microbiologicals (Cultures and Stocks)**
  Cultures and stocks of infectious agents and associated biologicals, including: cultures from medical and pathological laboratories: cultures and stocks of infectious agents from research and industrial laboratories; wastes from the production of biologicals; discarded live and attenuated vaccines; and culture dishes and devices used to transfer, incubate and mix cultures.
- **Human Blood and Blood Products**
  Waste human blood: products of blood: items saturated and/or dripping with human blood: or items that were saturated and/or dripping with human blood that are now caked with dried human blood; including serum, plasma, and other blood components; and their container, which were used in either patient care, testing and laboratory analysis or the development of pharmaceuticals.
- **Pathological Wastes and Human Bodily Fluids**
  Human pathological waste, including tissues, organs, and body parts and bodily fluids that are removed during surgery or autopsy, or other medical procedures, and specimens of bodily fluids and their containers. Cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, vaginal secretions, semen, pericardial fluid, and amniotic fluid from humans are all classified as infectious.
- **Sharps (including needles and blades)**
  Sharps that have been used in animal or human patient care or treatment or in medical, research, or industrial laboratories, including hypodermic needles, syringes, Pasteur pipettes, scalpel blades, razor blades, and needles with attached tubing, and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that were in contact with infectious agents, such as used slides or cover slips. Unused discarded sharps, hypodermic needles, suture needles, syringes and scalpel blades must also be disposed of as infectious waste.
- **Contaminated Animal carcasses and Bedding**
  Contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research.

- **Isolation Wastes**
  Biological waste and discarded materials contaminated with blood, excretion, exudates, or secretions from humans who are isolated to protect others from certain highly communicable diseases.
- **Recombinant DNA and Modified Genetic Materials**
  Under certain circumstances, viable organisms containing altered genetic material may present a potential for causing diseases or toxic effects. For example, host organisms and viruses are considered etiologic agents if they contain recombinant DNA when:
    1. The recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant parasite; or
    2. The recombinant DNA codes for toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth;
    3. The recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant parasite and has not been adequately characterized to demonstrate that it does not code for a factor eliciting human, animal or plant disease.

Stericycle is responsible for picking up infectious waste that is properly packaged and prepared by the generator.

*Infectious material/waste (or any other hazardous material) is **prohibited** from being placed into a refrigerator or freezer where food, medical supplies, and other sensitive materials are ordinarily stored.*

Important Note:** The biohazard waste disposal driver will not pick up infectious waste containers that are leaking, improperly packaged, unmarked, or otherwise mismanaged. **It is the responsibility of the generator to repackage any infectious waste containers that are found to be leaking or are improperly packaged.

10. DU Biohazard Waste Disposal Guidelines

Biohazard Waste Disposal Guidelines

A. Disposal of Waste
B. Recombinant or Synthetic Nucleic Acid Molecules and Transgenics
C. Liquid Biohazardous Waste
D. Solid, non-sharps biohazard waste
E. Tissue biowaste
F. Sharps
G. Insect Termination
H. Spills, Leaks, and Emergency Procedures

A. Disposal of BSL-1 Waste

Those cells and cell lines in BSL-1 and items that come in contact with these cells may be disposed of in the normal waste stream following appropriate treatment, (as described in specific sections below).
Segregate disposable solid waste into autoclavable plastic bags and decontaminate by autoclaving. You may dispose of decontaminated (BSL-1 only and no sharps) solid waste along with the Red Bin waste stream.

Disposal of BSL-2 Waste

BSL-2 waste and contaminated items must be disposed of as Regulated Medical Waste, (per directions in specific sections below), to maintain the safety of individuals who must handle the waste, as well as to comply with regulations promulgated by the Colorado Department of Public Health and Environment and OSHA.

Segregate disposable biohazard solid waste (e.g. intact plastic ware, gloves, paper) into red biohazard bags. Dispose of sharps waste (e.g. syringes, needles, Pasteur pipettes, broken glass) in sharps disposal containers. Bags and containers must be disposed of via approved Regulated Medical Waste transporter and treatment facility. Contact Environmental Health & Safety (303-871-4044) for more information. Regulated medical waste must not enter the normal solid waste stream. Decontaminate liquid wastes with disinfectant (e.g. a 1/10 dilution of household bleach for 30 min.), or in an autoclave, and dispose of in a sanitary drain followed by large amounts of water.

Red biohazard bags are used for the collection of non-sharps Biological waste at all points of origin. All biohazard bags meet the impact and tear resistance requirements of ASTM D-1709-91 and ASTM D- 1922-89, respectively. These biohazard bags are placed into leak proof, puncture resistant outer containers with lids (the Stericycle red bins). These containers are then removed by a licensed biological waste disposal firm. Clean containers and new bags are then replaced at each point of origin.

All Biological waste bags and sharps containers must be labeled with the generator's name and address. If the labeled bag is placed in a box or leak proof tub, the box or tub must also be labeled with the transporter's name, address, registration number and 24-hour telephone number. Items placed in the Biological waste bag or sharps containers are exempt from labeling when the waste bag itself is labeled.

Storage areas at each point of origin are located in buildings under contract with a licensed pest control firm. The storage areas have tile floors and can be easily decontaminated should a leak or spill occur. Each point of origin has controlled access away from student and pedestrian traffic.

A 30-day storage limit (90-day limit if stored below 45 degrees) is in force at each point of origin. For biohazard bags, the clock begins when the first waste item is placed in the receptacle. For sharps, the clock starts when the container is full and sealed. Waste containers should not have to be dated because pick-ups are pre-arranged with a licensed Biological waste disposal firm. The following information outlines the pick-up schedule for each storage area.

Generators, who do not receive pick up by the appointed time, should call 1-4044.

No Biological waste shall be manually compacted by the generator at the point of origin, or shall be removed from the receptacle or point of origin except by licensed Biological waste disposal firm.

Hazardous wastes should never be mixed with biohazardous wastes unless these are an unavoidable outcome of the generating process. In addition to the biohazard label, a label must be affixed to the bag that identifies the chemical hazard.

Radioactive wastes should never be mixed with biohazardous wastes (the sole exception being biological wastes coming from a process that used radioactive tracers). Selection of the tracer nuclide must be approved by the Radiation Safety Officer.

B. Recombinant or Synthetic Nucleic Acid Molecules and Transgenics

All materials, including animals, containing recombinant or synthetic nucleic acid molecules should be disposed of in accordance with the requirements of the NIH Guidelines. Recombinant/transgenic organisms should never be disposed of as regular waste. Specifically, Appendix G-II-A-1-c of the NIH Guidelines states that at Biosafety Level 1 and higher, all contaminated liquid or solid wastes must be decontaminated before disposal. Appropriate mechanisms for the decontamination of solid waste, including animal waste, includes, but is not limited to, incineration or autoclaving.

Recombinant and synthetic nucleic acid molecules are defined as:
(i) molecules that a) are constructed by joining nucleic acid molecules, and
b) can replicate in a living cell (i.e. recombinant nucleic acids);
(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified

C. Liquid Biohazardous Waste

Collection & Storage

Vacuum flasks should be stored in a non-breakable and leak-proof secondary container when not maintained inside a biosafety cabinet (BSC). Vacuum flasks must also be equipped with an overflow flask and/or HEPA filter on the line to protect vacuum lines in the event of a flask malfunction. Flasks should be discharged and cleaned weekly, or when they are half-full, whichever comes first, to prevent overflow and prevent growth of contaminants.

Treatment & Disposal of Liquid Waste

Treated liquid waste may be disposed of via the lab sink. Use a lab coat, gloves and splash goggles (or safety glasses with a face shield) when discharging waste to the drain. Use care to minimize generating “splash back” and thoroughly rinse the sink following waste discharge. Household bleach is 5 - 10 % sodium hypochlorite. According to Clorox, undiluted household bleach has a shelf life of six months - one year from the date of manufacture, after which bleach degrades at a rate of 20% each year until totally degraded to salt and water (exposure to sunlight or additional heat increases the degradation rate substantially), and a 10% bleach solution has a shelf life of 24 hours, so it must be prepared fresh daily. Treat liquid waste with standard bleach (10% in water solution) for 30 minutes, or, steam sterilize in the autoclave using the liquid cycle. Liquid waste may also be autoclaved and then disposed of via the sink. NOTE: If you will autoclave your waste, you should not pretreat with disinfectant, unless you use a disinfectant that is safe to autoclave based on information from the manufacturer. Bleach is not safe to autoclave. Remove or loosen caps before loading into the autoclave.

D. Solid, non-sharps biohazard waste

This includes lab items that have come in contact with viable biological materials that contain recombinant or synthetic nucleic acids, clinical specimens in a lab setting, and any lab materials that are regarded as potentially infectious.
Examples include:
•gloves used for biological material manipulations,
•disposable culture flasks, serological pipettes, pipette tips and well plates,
•waste items contaminated with blood in a manner that would present a personnel exposure risk (i.e., more than incidentally contaminated).

Collection & Storage

Collect waste in a solid-walled, leak-proof container lined with an autoclaveable biohazard bag. The container needs to have a lid and be marked with the biohazard symbol. The container must be closed when not waste is not being added.
Bench-top biohazard waste containers should meet the same criteria. Bags should be closed securely and transferred to the floor container when full.
Waste generated in a BSC should be collected in a biohazard container inside the BSC whenever procedures permit.
Collect serological pipettes separately (i.e., placed in pipette keeper boxes, in a dedicated bag, or in a box lined with a biohazard bag) in order to prevent bag puncture during the waste handling process.

Treatment & Disposal

Bags should be securely closed and transferred to a leak-proof secondary container in the designated biohazard waste pickup point in the lab. (Heavy bags should be double-bagged to further prevent leakage during the handling process.)

Bagged waste will be picked up by an outside vendor, place the securely closed bag in the vendor-supplied waste container for pickup and disposal. These waste bags are autoclaved, (or in some cases incinerated), for final treatment by the vendor.
NOTE: Bagged waste should always be stored in a secondary container designed to contain a leak including when being transported to autoclave facilities. (Double-bagging alone is not sufficient to contain a leak if the bags are ruptured.) Bagged waste treated on site must be treated using an established autoclave cycle that has been validated for effective sterilization of this waste.

E. Tissue biowaste

Collection & Storage

Tissue wastes should be collected:
•in sealable plastic bags marked with a biohazard symbol
•a biohazard bag that is securely closed.
Bagged tissues should be placed in a yellow secondary container that will contain a leak and then placed in a designated cold storage unit with a biohazard label.

Treatment & Disposal

Effective treatment of tissue waste requires incineration.

F. Sharps

This includes items which are sharp enough to puncture skin. Example devices include:
•needles & lancets,
•scalpels & razor blades,
•glass slides,
•glass Pasteur pipettes,
•biologically-contaminated broken glass, (uncontaminated broken glass may be collected/disposed in specially designated “empty/broken glass boxes).
This category also includes all sharps-associated medical devices (i.e., syringes).

Collection & Storage
These devices need to be placed into a sharps container immediately after use. Sharps containers shall be closable, puncture-resistant, red and/or or marked with biohazard symbol, and leak-proof on the sides and bottom.
NOTE: Sharps containers must be containers designed for that purpose. Cardboard boxes or repurposed food/beverage containers are not acceptable! Sharps container lids have a restricted access opening to prevent devices from being accessed once inside the container. Make sure that the lid is properly and completely installed before use. If the opening requires you to drop the device in vertically, it should be closed when the container is not in use. Keep the container free of visible contamination and store it in an upright position.

Treatment & Disposal
Permanently close the container when it is 2/3 - 3/4 full. (Filling beyond this level puts you at risk of a puncture wound involving potentially infectious pathogens). Place full containers in the red biohazard waste bins.

G. Insect Termination

All insects need to be terminated before final disposal. The preferred method is to place these in a -20 °C freezer until no longer viable. This can be done either before or after collection of closed primary containers in a biohazard bag for disposal.

In-lab Waste Collection & Storage
Insect waste should be collected in a biohazard waste bag that bears the biohazard symbol. The bag should be secured in collection container that is constructed of a rigid, leakproof, cleanable material. The container must be marked with the biohazard symbol. The container should be closed with a lid (constructed of the same material as the container) when wastes are not actively being deposited. All bottles and vials containing insects should be disposed of sealed with cotton plugs or other means to prevent escape from the primary container.

Disposal of Insect Waste
The bagged and treated insect waste can be disposed of in the same manner as other solid, non-sharps biohazard waste. (Transgenics must be autoclaved).

H. Spills, Leaks, Decontamination and Emergency Procedures

All spills of and surfaces contaminated with Biological wastes shall be decontaminated immediately upon discovery. Decontamination can be accomplished with a 1:10 mixture of ordinary household bleach and water (5,000 ppm chlorine). This can be used on solids and in liquids. Exercise caution, as chlorine gas can be irritating to the mucous membranes and eyes. Use in a well-ventilated area with protective gloves, gown and goggles. All contaminated materials should be treated as Biological waste and be disposed of in Biological waste containers. Liquids can be decontaminated as above and flushed down a sanitary sewer. Surfaces should be wet wiped with the bleach solution above and allowed to air dry to ensure sufficient contact time. Spill response supplies shall include absorbent material, biological waste bags, disinfectant and personal protective equipment and must be in place prior to any spill event requiring their use.

Upon discovery of a spill or leak:
1). Don protective gear such as gloves, safety goggles, lab coat or gown. 2). Use disposable sorbets such as pillows or granules (kitty litter) to soak up any fluids present. Dispose of as Biological waste. Solids and sharps should be scooped up or picked up with tongs, forceps, etc. Place sharps in sharps container.
3). Decontaminate the area with the bleach solution above or equivalent. Dispose of all material as Biological waste. Non-disposable items should be decontaminated with the bleach solution above.
Requests for assistance or reports of spills should be directed to EH&S at 1-7501. After-hours reports should go to Campus Safety at 1-3000. Spills or releases that may impact the environment or present a clear and imminent public health hazard should be reported to the Colorado Department of Public Health and Environment's 24-hour spill reporting hotline at 1-877-518-5608 and to the local response authorities where the spill or release occurred. Spills or releases in the sanitary sewer must also be reported to your local wastewater treatment facility.

Appendix 1

Pathogens should be handled and contained depending upon their biological characteristics. These characteristics or factors provide for an indication of the associated risk of exposure and the risk of disease of the host. The factors that are associated with the risk of exposure are the host's work activity, proficiency, age, sex, immune status and medications being used. The factors associated with the risk of disease to the pathogen include virulence, infectious dose, route of infection, toxigenicity, agent's host range, and the availability of effective preventive measures and treatment. Other factors which determine how susceptible one is to an infectious agent are the host's natural defense mechanisms and the chance for opportunistic infection.

The World Health Organization (WHO) has classified infective microorganisms by risk group. These are numbered 1-4 as follows:

- **Risk Group 1** no or low individual community risk

  A microorganism that is unlikely to cause human or animal disease, such as *E. coli* K-12 and *Saccharomyces cerrivisiae*.

- **Risk Group 2** moderate individual risk, low community risk

  A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious Infection, but effective treatment and preventative measures are available and the risk of spread of Infection is limited. Examples Include *Herpes* viruses, *HIV* (clinical work), *Varicella-Zoster* virus, and polioviruses.

- **Risk Group 3** high individual risk, low community risk

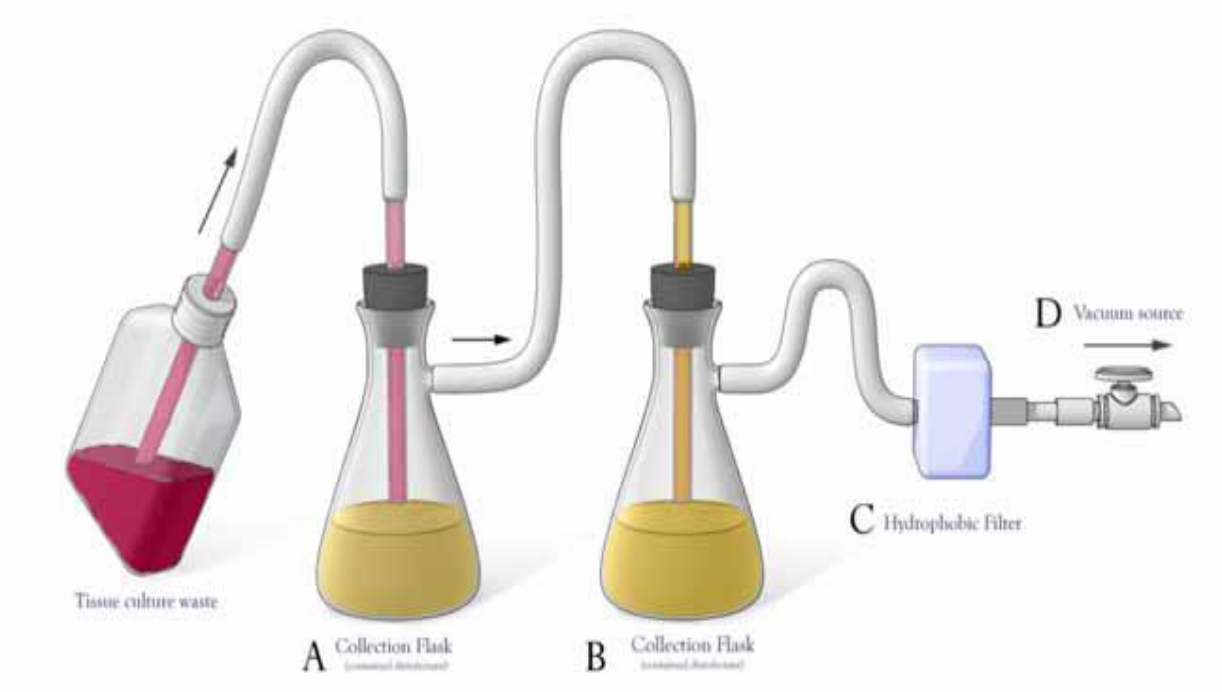
  A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected Individual to another. Effective treatment and preventative measures are available. Examples Include *HIV* (non-clinical work), *Mycobacterium tuberculosis, Coxiella burnetti, Brucella*, and *Hanta* virus.

- **Risk Group 4** high individual and community risk

  A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Examples include: *Ebola* and Hemorrhagic fevers.

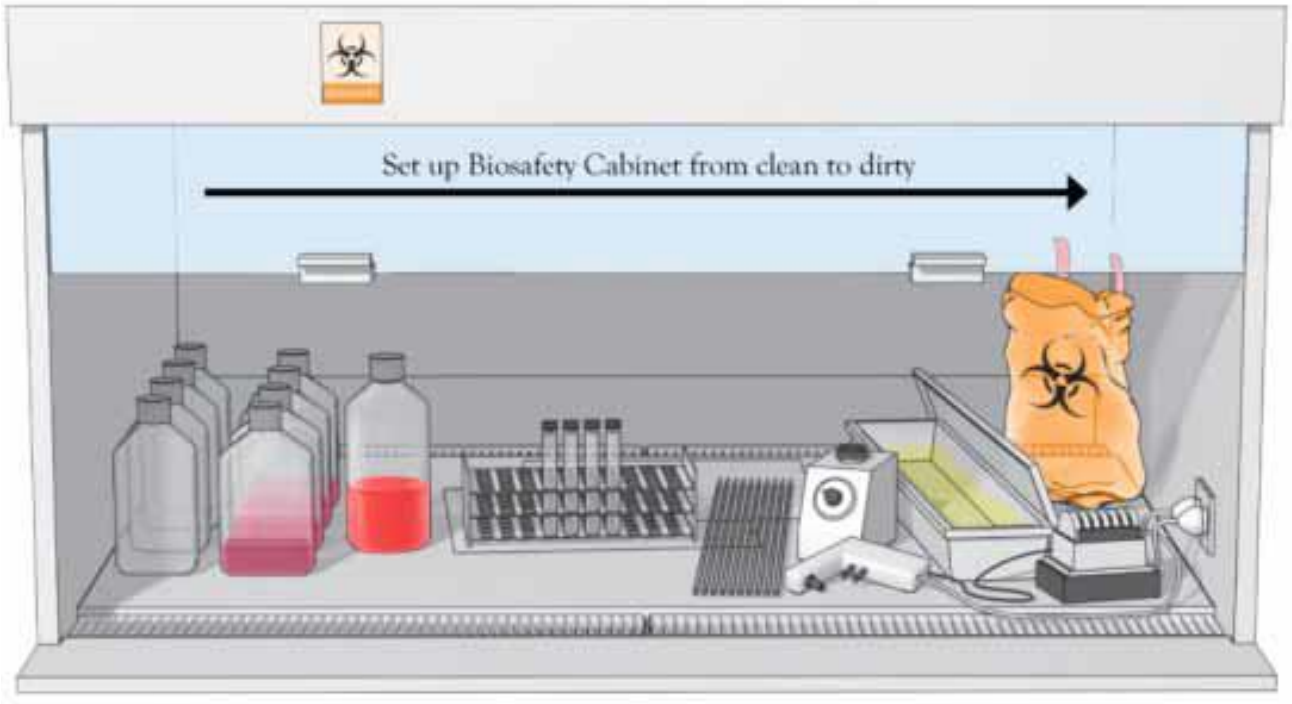
Appendix 2

Protection of Vacuum Lines

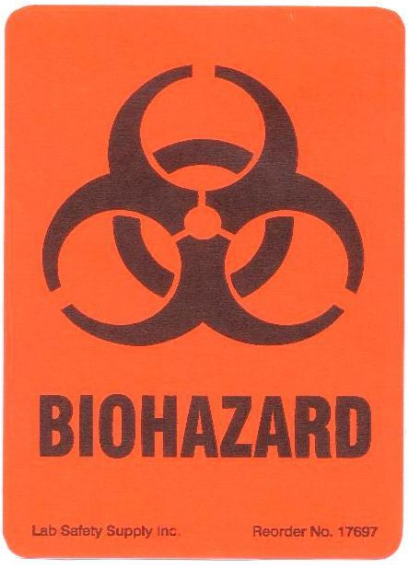


Appendix 3

Placement of Materials in BSC's



Appendix 4



Biosafety Level: _____

Principle Investigator: _____ Ext. _____

Immunization Required: Yes No

Special PPE Requirements: _____

Appendix 5
REFERENCE MATERIALS

Guidelines for Research Involving Recombinant DNA Molecules
http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition
https://www.cdc.gov/biosafety/publications/bmbl5/index.htm

OSHA Laboratory Standard
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10106

OSHA Hazard Communication Standard
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10099

OSHA Bloodborne Pathogen Standard
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

RISK GROUP DATABASE
https://my.absa.org/tiki-index.php?page=Riskgroups

Pathogen Safety Data Sheets and Risk Assessment
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

Chemical Laboratory Safety and Security: A Guide to Prudent Chemical Management
http://dels.nas.edu/global/bcst/Chemical-Management