Contents
SECTION 1 - INTRODUCTION .............................................................................4
SECTION 2 - ROLES AND RESPONSIBILITIES .............................................6
    General ........................................................................................................6
    Institutional Responsibility .......................................................................6
    Institutional Biosafety Committee ...........................................................7
    Biological Safety Officer (BSO) .................................................................8
    Principal Investigator (PI) .........................................................................8
    Responsibilities of Personnel During the Conduct of the Research .......... 10
SECTION 3 - General Biosafety .....................................................................10
    Workplace Practices and Planning ......................................................... 11
    Compliance ................................................................................................12
    Education and Training ...........................................................................15
SECTION 4 - TRAINING AND ENROLLMENT REQUIREMENTS ..............16
    Institutional Biosafety Committee Training ............................................16
    Online Biosafety Level (BSL) 1/BSL2 Training ......................................16
    Biological Safety Cabinet (BSC) Training ................................................16
    Blood Borne Pathogen (BBP) Training (Human Samples) ......................16
    Dangerous Goods 6.2 (Infectious Substance Shipping, Transporting, Receiving) and Dangerous Goods 9 Misc. (Dry ice and Genetically Modified Organisms/Micro-organisms) ............................................................. 17
    Mock BSL3/ABSL3 Laboratory Training ..................................................17
    General characteristics and clinical symptoms associated with infectious agents in the BSL3/ABSL3 laboratory .................................................. 18
    Barrier training inside the BSL3 ...............................................................18
    Select Agent Rules and Regulations ..................................................... 18
SECTION 5 – OCCUPATIONAL HEALTH PROGRAM ................................18
SECTION 6 – RISK ASSESSMENTS AND RISK GROUPS ......................19
    Classification of Biohazardous Agents ....................................................20
SECTION 7 – BIOLOGICAL SAFETY CONTROLS ....................................20
    Engineering Controls .............................................................................20
    Administrative Controls .........................................................................21
    Workplace Practices ..............................................................................21
    Personal Protective Equipment .............................................................21
SECTION 8- BIOSAFETY GUIDELINES AND BIOSAFETY LEVELS ........21
SECTION 1 - INTRODUCTION

Purpose and Scope
Colorado State University has developed this manual to provide information regarding policies and guidelines for a uniform biological safety program for work involving biological materials and recombinant DNA.

The provisions specified herein are applicable to all clinical, laboratory, research, service and support activities unless specifically changed, modified or waived by the Institutional Biosafety Committee (IBC). The guidelines and procedures described in this manual are derived from those developed by:
- CDC-NIH Biosafety and Biomedical Laboratories (BMBL): (http://www.cdc.gov/biosafety/publications/bmbl5/).

As used herein, the words "must," "will" or "shall" indicate mandatory requirements whereas the words "may", "should" or "recommend" indicate items for consideration as "good practice".

Further information related to specific safety programs, operations, and/or procedures can be obtained by contacting the Biosafety Officers (BSO) or Environmental Health Services (EHS). Chemical and radiological safety procedures are beyond the scope of this manual and can be obtained by contacting specialists at EHS or visiting the EHS website:
- www.ehs.colostate.edu.

Waivers and/or Modifications
Waivers from or modifications to controls or procedures specified herein may be granted to departments, agencies, projects or responsible persons, upon written request to the IBC/Biosafety Office/EHS, provided that:

Proposed procedures or controls provide, for the specific purpose for which waived or modified, operations at least as safe, secure and efficient as those specified herein.

Clinical operations involving known or suspected human, animal or plant biohazards will follow this handbook unless deemed medically inappropriate. Whenever medical decision overrules provisions herein, a responsible physician and/or veterinarian shall establish suitable safety precautions, on a case-by-case basis, to safeguard people, animals and/or plants.

Exceptions
An occasional waiver or modification necessary for the completion of an ongoing project may be granted with "acceptable" safety, security or efficiency when:
• Proposed procedures or controls are not a violation of law or an externally imposed directive which has not been formally waived or modified by the agency(ies) responsible.

• Proposed procedures and/or controls are not a violation of the University's Building and Fire Code or its standards unless a waiver or variance from this Code has been obtained from the Building Official (Director of Facilities Management) or the Code Variance and Appeals Board.

• Any applicable licenses or approvals from other control agencies of the University (Human Research or Radiation Control Committees, etc.) or by outside agencies (radioactive materials license, etc.) are obtained.

• Proposed procedures and/or specific controls are submitted to the Biosafety Office and approved by the IBC.

Procedures Not Controlled Herein
When no specific procedures or requirements are specified herein or otherwise required (by law, code, ordinance, standard, regulation, contract or grant agreement or other directive) compliance with a nationally or professionally recognized standard practice or prudent procedure acceptable to the IBC shall be deemed to satisfy the provisions of this manual. The IBC may however impose added requirements, restrictions or controls on specific projects as and when necessary for health, safety, environmental protection or the preservation of property. Such additional requirements are mandatory unless a specific waiver is granted.

Applicability of External Controls
Applicable laws, ordinances, codes, regulations, standards, contract guidelines or requirements of other directives imposed upon University activities are considered to be requirements of this handbook. However, where the provisions of this handbook provide better health, safety, environmental or property protection, the requirements in this manual apply. In case of conflict, or where other directives dictate violations of this manual’s provisions, the IBC shall be informed. The IBC will resolve such conflicts by changing or modifying this manual, by waiving or modifying requirements for specific project(s) involved, or help in obtaining waivers or variances from the other directive(s).

Supplements
Colleges, agencies, departments, principal investigators, laboratory supervisors and other responsible officials may supplement the provisions of this manual. Supplemental directives must be provided to the Biosafety Office, and a copy forwarded to the IBC, for final approval. Supplements shall follow the same format of this manual and should be published as page supplements with appropriate paragraphs referenced.

Supplements shall not delete any of this manual’s controls or requirements without prior approval from the IBC. Justification for such deletions will be required. Requests are to be processed as a waiver or modification to this manual through the biosafety office to
Institutional Biosafety Committee (IBC)
The IBC adopts procedures and controls specified herein with the advice and consent of the University's Vice President for Research (VPR) and Environmental Health Services (EHS) Biosafety Office. The IBC may, through majority rule, modify, change, delete or add to these requirements when and as necessary after appropriate reviews, hearings and approval.

The IBC shall review suggested changes to this manual and all requests for modification or waivers to its procedures and requirements prior to approval.

The IBC shall review supplemental procedures and requirements developed in support of this manual by departments, principal investigators, project leaders and/or other responsible persons. Such supplements may be approved or modified for use by only the submitting person or agency (and their subordinates), or the IBC may adopt or modify and then adopt them for overall application and change in this manual accordingly.

SECTION 2- ROLES AND RESPONSIBILITIES

General
The safe conduct of experiments involving biological materials and recombinant DNA depends on the individual conducting such activities. It is not possible to anticipate every possible situation, and motivation and good judgment are the key essentials to protection of health and the environment. The NIH and BMBL guidelines are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should, and must, be implemented. No guideline will ever be complete or final since all conceivable experiments involving biological materials and/or recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the institution and those associated with it to adhere to the intent of this manual and to the NIH and BMBL Guidelines as well as to their specifics. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research.

Institutional Responsibility
Each institution is responsible for ensuring that research is conducted in full conformity with the provisions of federal, state, and local regulations and guidelines; contract guidelines or requirements of other directives imposed upon University activities. In order to fulfill this responsibility, the institution shall institute policies and procedures to ensure compliance. The President of CSU is ultimately responsible for all environmental health and safety issues and exercises this authority by delegating the charge for ensuring safe practices and compliance through the established chain of authority: Vice President
for Research, Institutional Biosafety Committee, Deans, Department Chairs, Principal Investigators, supervisors, and individual employee.

Additionally, each institution conducting or sponsoring recombinant DNA research, which is covered by the *NIH Guidelines*, is responsible for ensuring that the research is conducted in full conformity with the provisions of the *NIH Guidelines*. In order to fulfill this responsibility, the institution shall:

- Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the NIH Guidelines. As part of its general responsibilities for implementing the NIH Guidelines, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the NIH Guidelines. Such procedures may include: (i) statements formulated by the institution for the general implementation of the NIH Guidelines, and (ii) any additional precautionary steps the institution deems appropriate.

- Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in The NIH Guidelines, Section IV-B-2-a, and carries out the functions detailed in Section IV-B-2-b.
  
  o NIH mandated IBC Requirements:
    
  
  o NIH mandated IBC Functions:
    

**Institutional Biosafety Committee**

**Membership**

The IBC is appointed by the Vice President for Research and represents a collection of faculty, staff and community members with a diversity of expertise and knowledge related to recombinant DNA, infectious agents, toxins, animal models, plant models and biological safety.

**Responsibility**

The IBC is responsible for the review, approval, and oversight of all research projects involving potentially biologically hazardous and recombinant DNA activities. The IBC also provides policy recommendations to the Office of the Vice President for Research in order to ensure compliance with federal, state, and local regulations and guidelines. The IBC has the authority to implement operational changes and to limit or suspend research that is not in compliance with the CSU Biosafety Program. The institution is ultimately responsible for the effectiveness of the IBC, and may establish procedures that the IBC shall follow in its initial and continuing review and approval of applications, proposals, and activities.
More information about the CSU IBC can be obtained at:
  •  [http://web.research.colostate.edu/ricro/ibc/about.aspx](http://web.research.colostate.edu/ricro/ibc/about.aspx)

**Biological Safety Officer (BSO)**

CSU has one Biosafety Officer (BSO), and one or more Associate or Assistant Biosafety Officers. The Biological Safety Officer shall be a member of the IBC. Working under the Department of Environmental Health Services, all BSOs are the primary point of contact for biosafety matters.

The Biological Safety Officers’ duties include, but are not limited to:
  • Monitoring compliance with Federal, State and University biosafety policies and procedures, including inspections/audits to ensure that laboratory standards are rigorously followed;
  • Reporting to the IBC and the institution any significant problems, violations of the NIH Guidelines, local or federal laws, or other CSU established policies, and any significant research-related accidents or illnesses of which the BSO becomes aware;
  • Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving biological and recombinant DNA research;
  • Providing advice on laboratory security;
  • Providing technical advice to Principal Investigators and the IBC on research safety procedures;
  • Developing and conducting appropriate training programs for safe handling, transport, and disposal of biological hazards;
  • Investigating accidents involving biological agents that have the potential for personnel or environmental exposure and providing assistance to prevent future occurrences.

**Principal Investigator (PI)**

On behalf of the institution, the PI is responsible for the safe operation of research activities and full compliance with the established Federal, State and CSU policies and procedures. Responsibilities include, but are not limited to:
  • Reporting any significant problems and/ or violations of the NIH Guidelines, Select Agent Program, Federal and State laws, or CSU policies/procedures, or any significant research-related accidents and illnesses to the BSO and IBC, and also to the following, where applicable: Building Directors/ Proctors, Greenhouse or Animal Facility Directors, EHS and other appropriate authorities (if applicable). These authorities will report to the NIH Office of Biological Activities (OBA).
If working with recombinant DNA, all responsibilities as described in the NIH Guidelines must be met, which include timely reporting of incidents or problems to the appropriate authorities.


- Report any new information bearing on the *NIH Guidelines* to the IBC and to NIH/OBA

- Adequate training in good microbiological techniques;

- Adherence to approved emergency plans for handling accidental spills and personnel contamination;

- Compliance with shipping requirements for biological materials and recombinant DNA molecules (see Appendix H in the *NIH Guidelines, Shipment*, section for shipping requirements for technical recommendations: [http://oba.od.nih.gov/oba/rac/Guidelines/APPENDIX_H.htm](http://oba.od.nih.gov/oba/rac/Guidelines/APPENDIX_H.htm)).

**Submissions by the Principal Investigator to the Institutional Biosafety Committee**

The Principal Investigator shall:

- Make an initial risk assessment in order to determine the required levels of physical and biological containment in accordance with the this manual, CSU policies and procedures, Select Agent Program (if select agents will be used) and *NIH Guidelines* (if recombinant DNA will be used);

- Select appropriate microbiological practices and laboratory techniques to be used for the research, as appropriate to the organism and the research being performed;

- Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, III-B, III-C, III-D, or III-E (*Experiments Covered by the NIH Guidelines*, [http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm#_Toc7261559](http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm#_Toc7261559)), to the IBC for review and approval or disapproval; and

- Remain in communication with the IBC throughout the conduct of the project.

**Responsibilities of the Principal Investigator Prior to Initiating the Research**

The Principal Investigator shall:

- Receive IBC approval for all projects and biological agents used in the scope of their research;

- Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

- Assure appropriate instruction and training of staff, including but not limited to: (i) required institutional training modules; (ii) the practices and techniques required to ensure safety, and (iii) the procedures for dealing with accidents;
Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations, medical clearance or serum collection).

**Responsibilities of the Principal Investigator During the Conduct of the Research**

The Principal Investigator shall:

- Ensure that the required safety practices, techniques and PPE are provided and employed;
- Correct work errors and conditions that may result in the release and/or exposure of biological or recombinant DNA materials;
- Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment (e.g., purity and genotypic and phenotypic characteristics);
- Comply with reporting requirements (e.g., the Select Agent Program and human gene transfer experiments conducted in compliance with the *NIH Guidelines*—see Appendix M-I-C, *Reporting Requirements*);
- Complete a final lab audit and remove all biological material/close out lab prior to leaving the University or moving to a different lab space.

For information regarding the roles and responsibilities of NIH, OBA and the Recombinant DNA Advisory Committee (RAC) please refer to the following website:


**Responsibilities of Personnel During the Conduct of the Research**

The Individual performing the research shall:

- Act carefully and prudently, and to adhere to CSU policies and guidelines outlined in this manual. Since not all circumstances can be foreseen or described in this manual, it is the responsibility of the individual to use good judgement and to adhere to the INTENT of the guidelines as well as the specifics.
- Be familiar with and know the hazards of materials or substances used; the guidelines, rules or regulations pertaining to their activities, and to carefully observe protocols.
- Have appropriate training as required by Federal, State, and CSU policy and must consult with their supervisors to receive appropriate instruction and training specific to the handling and disposal of biohazardous materials utilized in their workplace and accident management procedures.
- Correct any unsafe conditions or practices observed within his/her ability or authority, and/or to report them to the responsible PI, supervisor, and the BSO. Pregnant women and persons who are immune-compromised or have other health conditions are encouraged to consult with their supervisor, Poudre Valley Hospital Occupational Health Services, or physician of choice concerning potential risks and management of these risks.

**SECTION 3 - General Biosafety**

Biosafety consists of a four part integrated program and includes: 1) Engineering and

Engineering Controls
The engineering and maintenance concept considers both people and their workplace conditions and includes: 1) the selection and monitoring of activities, areas, facilities and equipment; 2) the design, construction, renovation or modification, inspection, certification and maintenance of facilities and equipment; 3) the destruction or safe disposal of biological waste; and 4) the decontamination of facilities and equipment no longer to be used in biological activities.

Design and construction of new facilities and modifications of existing facilities for biological activities shall conform to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Guidelines (BMBL), the NIH Guidelines for Recombinant DNA Research and other criteria specified by the Federal Government and the Biosafety Office in addition to requirements of the University's Building and Fire Code. The most current edition of these guidelines will be used. The BSO shall review and the IBC will approve such design and final completion of work before biological projects are started in new or remodeled facilities.

Maintenance and repairs to existing biological facilities or equipment shall not violate containment requirements specified for the area. Containment integrity shall be certified by a qualified inspector before new construction, remodeling or renovation is considered complete.

NOTE: All other maintenance and repair activities in biological areas shall however, be jointly scheduled between Facilities Management and persons in charge of the biological area/activity so that dangers to personnel, projects and/or the environment can be controlled.

Redundant containment controls for biological agents shall be used. The IBC also has authority to impose additional and/or other redundant controls to contain biological agents whenever deemed necessary.

Workplace Practices and Planning
The BSO and IBC review all biological activities through inspections/audits of both work practices and the work environment.

Emergency Action Plans for individual facilities should be made available to employees and to emergency response personnel so they can better control the emergency, decontaminate themselves and/or their equipment and/or receive any needed prophylactic treatments. It is expected that one or more knowledgeable persons from the facility involved, the PI or proctor, will be available to assist in, or advise on response actions and to check the safety of the incident area along with emergency response personnel before the area/facility is returned to normal usage or turned over to repair/demolition personnel. Material Safety Data Sheets (MSDSs) or
similar information regarding biological materials or substances involved or possibly encountered must be made available to personnel.

**Compliance**

All persons involved in biological activities, whether faculty, staff or students, paid or unpaid, full or part time, are expected to always act carefully and prudently and to conform to this manual. Each person is expected to be familiar with and know the hazards of materials or substances used; the guidelines, rules or regulations pertaining to their activities; and carefully observe procedures and protocols. Each person is also expected to correct any unsafe conditions or practices observed within his/her ability or authority, and/or to report them to the responsible PI, supervisor, and the BSO.

The CSU Biosafety Office will conduct audits biannually, or as otherwise required, of each laboratory to ensure compliance with the procedures and protocols of this manual. Audit reports will document violations and be directed to the PI. The results of these audits will be documented and kept on file with the Biosafety Office. Any significant problems will be reported to the IBC. Redundancy in ensuring observance of safe laboratory practices, just as in containment, is needed because individuals may subconsciously develop unsafe practices or fail to recognize unsafe conditions. For this reason, all PIs and others supervising or overseeing biological activities are expected to closely and thoroughly inspect the work, work practices and work conditions of all individuals in their laboratories and expeditiously correct any unsafe conditions and/or practices observed.

**Key Components for Compliance:**

1. **Standard Operating Procedures.**
   a. PIs and others proposing work with biological materials must develop detailed procedures for those activities and see that they are followed when approved.
   b. When required by directives or when required by this manual, these procedures are to be submitted to the IBC and/or the BSO. The IBC and/or BSO shall review hazards and procedure(s) involved and, as appropriate, approve, direct or suggest changes. BSO approvals will be submitted to the IBC for comment and review.

2. **Audits and Oversight.**
   a. The IBC, with the assistance of subcommittees or specialists, will oversee and ensure compliance with the provisions of individual project protocols, legal requirements, this manual and University Policy.
   b. The BSO will perform lab audits. Reports shall be provided to the PI/supervisor involved. A summary of the audits will be provided to the IBC. Corrective actions, to the satisfaction of the IBC, are to be taken for each deficiency noted.
   c. Department heads are accountable for the biosafety of subordinates and their activities. They shall ensure security and quality controls necessary
for biological activities within their jurisdiction. They are encouraged to use assistance from the BSO, the IBC and EHS if needed. Action needs to be taken within 3 months of the audit, or sooner for items that pose imminent threat to safety.

3. Requirements for Visitors and Untrained Personnel

a. Access to areas containing biological agents is restricted and/or limited for: visitors (including delivery and trades personnel), any person under the age of 16 years and non-immunized persons to areas where immunizations are required. This restriction is to protect the individual, equipment, supplies, work in progress and experimental animals or plants from contamination. Minimum requirements are specified by the type of activity within individual chapters of this manual. (See Sections 4, 5, and 7)

b. Restricted access areas shall be fully identified by keycard panels/ pads and/or signs (which also aid emergency response personnel). At a minimum such signs shall provide emergency response personnel with names of knowledgeable persons or contacts and information on hazards (chemical toxicity, flammability, reactivity, radiation hazard, biohazard and animal hazards) within the area. This enables emergency response personnel to protect themselves, rescue people and/or better contain the emergency.

c. Copies of MSDS’s, when requested, shall be provided to any emergency response personnel by the PI/ supervisor.

d. Contractor personnel must be trained and informed of the biological hazards to which they could potentially be exposed and shall not work on or in biohazard areas unless prior decontamination has been satisfactorily accomplished. If any research activities are to continue while contractor personnel are in the area or the equipment is not able to be decontaminated, adequate isolation provisions and PPE shall be used to protect both personnel and research.

4. Biological Laboratories

a. All work in biological laboratories is to conform to requirements of this manual, applicable NIH/ CDC/ USDA "Guidelines" and any other directives or guidelines determined applicable by the IBC for the specific activity.

b. Any renovations of facilities necessary for biological activities shall conform to requirements of this manual (including NIH and BMBL Guidelines"), and have prior approval by the Biosafety Office and CSU Facilities Services with overview by the IBC.

c. When a biological activity involves radioactive materials, ionizing radiation, human or human tissue research studies, or controlled substances or dangerous drugs, approval from the applicable control
agency (internal and/or external) is required in addition to approval by the IBC.

d. Approval of Projects Using Biological Agents. PIs must submit for approval an Agent Approval Request Form (AARF) and/or a Project Approval Request Form (PARF) for projects using biological agents. Approval must be received before research can commence.

e. Other

i Diagnostic, Analytical and Clinical Laboratories. These labs receive specimens with requests for a variety of diagnostic and clinical support services. Pertinent information, such as a history or other findings which may be suggestive of infectious etiology or specific chemical problems, may be unavailable. Specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputum samples submitted for "routine", acid-fast and fungal cultures), and/or chemical evaluation (identity, contaminants, toxicity, etc.).

(1) It is the responsibility of the laboratory director/supervisor with assistance from the Biosafety Office and approval of the IBC, to establish standard laboratory procedures which address the issue of the potentially infectious hazard of specimens.

(2) Except in extraordinary circumstances (e.g., suspected plague, etc.) the initial processing of clinical specimens and identification of isolates can be safely conducted using a combination of practices, facilities and safety equipment described as BSL-2. Biosafety Cabinets (BSCs) (Class I or II) should be used for initial processing of clinical specimens when the nature of the test requested or other information suggests that an agent that is readily transmissible by infectious aerosols is likely to be present. Class II BSCs are also used to protect the integrity of the specimens or cultures by preventing contamination from the laboratory environment. Laboratory chemical fume hoods can be used when and as necessary depending upon the type of analysis desired for chemical or specimen analysis (e.g, inactivated or fixed tissue).

(3) Segregating laboratory functions and limiting or restricting access to laboratory areas are the responsibility of the laboratory director/supervisor.

ii Field Studies. Field studies are biological studies that occur in the natural environment. Personnel should consult with the Biosafety Office for a risk assessment prior to conducting a field study. Field studies also include any intentional release of a genetically modified or artificially engineered living agent or their toxins to the environment, or the use of a chemical potentially capable of changing the environment for some biological control purpose (e.g., pesticide); these studies shall be accomplished only with prior approval of the
IBC. NOTE: Most studies of this nature will also require prior approval of USDA and/or Environmental protection Agency (EPA).

(1) Re-entry times for plant fields used for agricultural studies must be the longest time established by EPA, USDA and/or the IBC. Persons performing experimental work in fields prior to authorized re-entry times must be fully equipped with personal protective equipment as is necessary for initial application and approved by the IBC.

**Education and Training**

All persons should meet the minimum requirements, as indicated by the BSO and guidelines issued by the BMBL and *NIH recombinant DNA Guidelines* for the area or activity involved. Education requirements are developed by the Biosafety Office (BSO) with approval of the IBC. Individuals directly involved with biological agents must be formally trained for their specific tasks by the PI and the Biosafety Office.

Lab specific training is provided by the PI. The PI may assign a lab manager to train an individual; however, the PI is ultimately responsible for ensuring and documenting that training has occurred. All persons working with or around biological agents must be instructed in the specific hazards of the agents and procedures, methods to avoid those hazards, and emergency procedures.

All persons working with or around biological agents must:

- Be instructed in standard and special microbiological practices associated with their Biosafety level, entry and exit control procedures; the meanings of the various signs, controls and lab procedures used; emergency procedures applying to their work activities and area, recognition and prevention of dangerous situations and/or exposures, and the symptoms (acute and chronic) of possible exposures.
- Receive documented training in basic biosafety controls; applicable directives (including use of this manual); and specific methods and requirements of their work and work area.
- Complete the Occupational Health Program, Risk Assessment Form, and enroll in surveillance programs as deemed necessary. The Occupational Health Risk Assessment Form is required to be updated annually and/or when changing job responsibilities, and can be found at:
  - [http://www.ehs.colostate.edu/WOHSP/Home.aspx](http://www.ehs.colostate.edu/WOHSP/Home.aspx).
- Follow the policies of the CSU Respirator Program, where applicable.

In addition, awareness training shall be provided to maintenance personnel. The extent of the training will be determined by the Biosafety Officer with approval by the IBC in accordance with the potential exposure.
Training certifications/documentations will be maintained as required by the Biosafety Office and additional education or training requirements are to be imposed as stated.

SECTION 4-TRAINING AND ENROLLMENT REQUIREMENTS

CSU Biosafety training addresses a multitude of requirements established by Occupational Safety and Health Administration (OSHA), NIH, CDC, USDA, Office of Laboratory Animal Welfare (OLAW) and other safety, health and environmental regulators. Access to the training, and a summary of training requirements by work classification, are located on the following Environmental Health Services websites:

- Training enrollment: https://wsnet.colostate.edu/cwis86/WTrainReg/ClassSignUp.aspx

**Institutional Biosafety Committee Training**

This training is required for all individuals submitting agent and project approvals to the IBC. This training covers the NIH recombinant DNA guidelines.

**Online Biosafety Level (BSL) 1/BSL2 Training**

This training outlines the requirements and safety practices for working in a BSL1 and/or BSL2 laboratory. Training is required every 2 years.

**Biological Safety Cabinet (BSC) Training**

Training includes discussion of the differences between the biosafety levels and also:
- Differences between hoods and BSCs;
- The different classes of BSCs;
- Basic principles of BSC functions;
- Proper use, cleaning, and maintenance of the BSC.

**Blood Borne Pathogen (BBP) Training (Human Samples)**

CSU requires employees with occupational exposure to blood or other potentially infectious material (including working with human cell lines) to receive blood-borne pathogen training at the time of assignment to tasks where occupational exposure may take place and at least annually thereafter. Additional training must be provided and documented when changes affect employees' occupational exposure. This training is documented, and minimally includes:
- A general explanation of epidemiology of and symptoms of blood-borne diseases
- Modes of transmission of blood-borne pathogens
- An explanation of the CSU Exposure Control Plan and how to get a copy of the plan
- Appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials.
- Use and limitations of engineering controls, work practices, and personal protective equipment.
- Information of hepatitis B vaccine, including efficacy, safety, etc.
Appropriate actions in emergencies with blood or other potentially infectious materials.

The procedure to follow if an exposure incident occurs.

Post-exposure evaluation information.

**Dangerous Goods 6.2 (Infectious Substance Shipping, Transporting, Receiving) and Dangerous Goods 9 Misc. (Dry ice and Genetically Modified Organisms/ Microorganisms)**

This Training meets the requirements for International Air Transport Association (IATA)/ Department of Transportation (DOT) 6.2 Dangerous Goods training and is required if individuals receive this training at CSU. Training validation is by successful completion of shipping scenario exercises that include evaluation and classification of substances to be shipped, labeling, packaging, and preparing shipping documentation. A certificate of training is printed following successful completion of the training. Training includes:

- Shipping regulations;
- Classification of infectious substances;
- Selection of proper packaging for shipping;
- Proper labeling of packages;
- Documentation required for shipping.

**Mock BSL3/ABSL3 Laboratory Training**

All persons are required to have this training prior to working in a BSL3/ABSL3 laboratory. Persons are required to schedule this training with the Biosafety Office. Training includes:

- Discussion of the biosafety levels, biosafety controls and biological risk groups.
- Biosafety practices and techniques;
- Gate and building entry;
- Barrier entry and exit;
- Putting on and taking off personal protective equipment (PPE);
- Use of N-95 respirators and PAPRs;
- Proper use and cleaning of a biosafety cabinet
- Minimization of aerosol generation;
- Select agent records, if applicable;
- Emergency response procedures – including barrier exit in case of fire; spill response (inside and outside of the BSC); medical emergency response to incapacitated person, needle stick injury, cut or puncture; animal escape, if applicable; loss of power; loss of barrier integrity; PAPR malfunction.

All training procedures involve “hands-on” training in the Mock BSL3/ABSL3 laboratory. Training validation is by completion of tasks on a checklist, by signature of the trainer and trainee, and a quiz. Annual refresher training is required.
**General characteristics and clinical symptoms associated with infectious agents in the BSL3/ABSL3 laboratory**

This online training is required by all persons prior to working in the BSL3/ABSL3 laboratory. This training includes an overview of:

- Risks associated with working with each agent;
- Transmission;
- Clinical symptoms;
- Prevention (including vaccination) of disease;
- Available treatments

Training validation includes successful completion of an exam. This training requires an annual refresher.

**Barrier training inside the BSL3**

This training is required for all persons prior to working alone in the BSL3/ABSL3 laboratory. It is the responsibility of the PI to ensure that persons under their supervision are adequately trained, and the training is documented, prior to any person being allowed to work independently in the barrier. Lab specific BSL3/ABSL3 procedures, including those covered in Unit 3, will be reviewed in the BSL3/ABSL3 laboratory setting. Training validation is required. Observation by a Biosafety Officer can and will be performed at any time, or upon request.

**Select Agent Rules and Regulations**

This training is required annually for all persons with select agent approval. This training must be taken prior to working in select agent BSL2, BSL3/ABSL3 laboratories. Topics that are discussed include:

- Entity registration for Select Agents and Toxins (SAT);
- Security risk assessments;
- Amendments;
- Requirements, including training, for working with SAT;
- Animals as Select Agents;
- Loss, theft, or release of a Select Agent-Reporting Requirements;
- Inter- and intra-entity transfer of SAT;
- Inventory control.

Training validation is by successful completion of a quiz acknowledging review and understanding of the training material.

**SECTION 5 – OCCUPATIONAL HEALTH PROGRAM**

All CSU employees working with biological agents are required to be enrolled in the Occupational Health Program and complete the Risk Assessment Form annually:

- [http://www.ehs.colostate.edu/WOHSP/Home.aspx](http://www.ehs.colostate.edu/WOHSP/Home.aspx)
The Occupational Health Program provides medical surveillance for biological agents, chemicals, respirator use, noise and exposure to animals. This includes vaccination of researchers with approved vaccines, tuberculosis surveillance and medical clearance for respirator use. The Occupational Health Risk Assessment Form is required to be updated annually and/or when changing job responsibilities.

**N-95 Fit testing**
Respirator fit testing (including N95 respirators) is required annually for individuals that are required to wear these respirators. Testing is performed using quantitative analysis.

**SECTION 6 – RISK ASSESSMENTS AND RISK GROUPS**

The PI is required to make an initial risk assessment for each project based on the hazards associated with the project (e.g. specific hazards such as high concentration of agent, aerosolization, animal models, use of sharps) and the hazards associated with the biological agent being worked with (e.g. low infectious dose, ease of transmission, treatment options available).

Fact sheets about infectious agents can be found:
- CSU BSL3 Infectious Agent Fact Sheets

To prepare a risk assessment, individuals need to be familiar with:
- The organism:
- Risks associated with working with each agent;
- Transmission;
- Clinical symptoms;
- Prevention (including vaccination) of disease;
- Available treatments
- The definition of Biological Safety Levels (BSL) 1, 2, 3 and 4;
- The lab procedures to be performed and associated risks;
- Engineering controls, administrative controls, workplace practices and personal protective equipment (PPE) that can be used to mitigate the identified hazards.

Once these factors are analyzed, one can assign the appropriate biosafety level and biosafety practices associated with the research.
**Classification of Biohazardous Agents**

Guides to assist in this assessment can be found in the National Institute of Health (NIH) Guidelines, the World Health Organization (WHO), and the BMBL Section II. These guidelines provide an introduction to risks associated with an organism. Risk Group assignment and Biosafety Level (containment) are not synonymous.

- **Risk Group 1** are agents not associated with disease in **healthy** humans or animals.
- **Risk Group 2** are agents associated with human or animal disease which is **rarely** serious and for which preventive or therapeutic interventions are **often** available.
- **Risk Group 3** are agents that are associated with **serious or lethal human or animal disease** for which preventive or therapeutic interventions **may be** available (high individual risk but low community risk).
- **Risk Group 4** are agents that are likely to **cause serious or lethal human or animal disease** for which preventive or therapeutic interventions are **not usually** available (high individual risk and high community risk).

Examples of organisms that have been classified into particular risk groups are provided in **APPENDIX 1** of this manual.

To determine the current classification for an organism of interest, please consult the document *Classification of Human Etiological Agents on the Basis of Hazard*:

- [http://www4.od.nih.gov/oba/rac/guidelines_02(APPENDIX_B.htm](http://www4.od.nih.gov/oba/rac/guidelines_02(APPENDIX_B.htm)

**SECTION 7 – BIOLOGICAL SAFETY CONTROLS**

Publications of the occurrences of Laboratory Acquired Infections (LAIs) have provided invaluable resources for the research community. Historical accounts have raised awareness about the hazards and health risks of infectious microorganisms to lab workers and researchers.

Individuals and groups have published suggested practices and methods that help prevent or minimize the risks associated with biological agents. These practices and methods are known as biosafety controls.

To control hazards and risks associated with biological material, engineering controls have been developed, administrative controls became regulated, safety practices have been implemented and personal protective equipment has been developed.

**Engineering Controls**

Engineering controls are equipment in the lab that is provided for safety. Examples of safety equipment may include:

- Biological Safety Cabinets
- Airtight o-ring sealed rotors for centrifuges
- Mechanical Pipette Aids
- Secondary transport containers
- Autoclaves
• Puncture resistant sharps containers

**Administrative Controls**
Administrative controls are rules and regulations mandated or required for safety and may include:
• Occupational Health Program
• Medical surveillance
• Vaccinations
• Training
• Background checks
• OSHA standards
• Certifications
• Grant requirements

**Workplace Practices**
Workplace practices are plans, policies, procedures and protocols implemented for safety and may include:
• Exposure Control Plan
• Sharps policy
• Biosafety Manual
• Equipment manuals
• Lab specific procedures

**Personal Protective Equipment**
Personal Protective Equipment (PPE) is the protective clothing worn for safety and may include:
• Close toed shoes
• Lab coat, surgical gown or tyvek suit
• Face mask
• Safety glasses, goggles or face shield
• Gloves
• Chemical Apron

Each PI and research group should identify the hazards associated with their project and the biological agent being worked with and use these references and controls to minimize the risk.

For further information and references, please see:

**SECTION 8- BIOSAFETY GUIDELINES AND BIOSAFETY LEVELS**
Colorado State University adheres to the procedures outlined in the most current edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL) by the U.S. Department of Health and Human Services, National Institutes of Health and CDC, and the NIH Guidelines for Research Involving Recombinant DNA Molecules. A copy of these publications can be obtained online at:


A biosafety level consists of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities which allow safe handling of a particular organism. The PI/ Lab Director is specifically and primarily responsible for assessing risks and for identifying and applying the recommended biosafety levels. The essential elements of three of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are directly derived from the BMBL:

- [http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_IV.pdf](http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_IV.pdf) and are summarized below.

The biosafety levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment. Level Four is not discussed here since we do not have BSL4 facilities at Colorado State University.

**Standard Microbiological Practices to be Followed at All Biosafety Levels**

Laboratories under all biosafety levels are required to adhere to the following Standard Microbiological Practices:

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
   a. Precautions, including those listed below, must always be taken with sharp items.
      i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
ii Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

iii Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Procedures should be performed carefully to minimize splashes and/or aerosols.

7. Work surfaces must be decontaminated with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.

8. All cultures, stocks, and other potentially infectious materials must be decontaminated before disposal. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
   a. Must be placed in a durable, leak proof container and secured for transport.
   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign will include the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

11. The PI/ laboratory supervisor must ensure and document that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals with these conditions are encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.
Special Practices, Safety Equipment, and Laboratory Facility Requirements

CSU Undergraduate Labs

- Only low hazard and closely supervised biological activities are to be accomplished in undergraduate academic laboratories unless specific approval from the IBC has been obtained.
- Only work at a Biosafety Level 1 (BSL-1) or Biosafety Level 2 (BSL-2). Infections agents which are suitable for work at these biosafety levels shall be used.
- No person should be permitted to work alone in an undergraduate laboratory. A "buddy system" shall be used whenever feasible.
- Standard microbiological practices will be used. These include practices such as appropriate dress and personal protective equipment; no food, drink, smoking, chewing, etc. while in the laboratory; the use of mechanical pipettors; containment controls such as laboratory hoods and BSCs; personal hygiene practices; centrifugation procedures, aerosol control; and operations and storage practices.
- Research activities are expected to set a proper example in biosafety for all to follow. Persons involved will be evaluated by their instructors on their conformance to biosafety requirements and practices.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immune-competent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by an appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by an individual with training in microbiology or a closely related science. The following standard practices, safety equipment, and facility requirements apply to all laboratory personnel.

Laboratory Biosafety Level Criteria – Biosafety Level 1

1. Special Practices:
   a. None required.
2. Safety Equipment (Primary Barriers and Personal Protective Equipment)
   a. Special containment devices or equipment, such as BSCs, are not generally required.
   b. Laboratory coats, gowns, or uniforms are required to prevent contamination of personnel. Refer to CSU Laboratory Guidelines and the most recent version of the BMBL. Protective eyewear should be worn
when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

c. Gloves must be worn to protect hands from exposure to hazardous materials.
   i. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available.
   ii. Wash hands prior to leaving the laboratory.
   iii. In addition, BSL-1 workers should:
      1. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
      2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
      3. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

3. Laboratory Facilities (Secondary Barriers)
   a. Laboratories should have doors for access control.
   b. Laboratories must have a sink for hand washing.
   c. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not allowed.
   d. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   e. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   f. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
   g. Laboratories windows that open to the exterior should be fitted with screens.
Laboratory Biosafety Level Criteria – Biosafety Level 2

All standard microbiological procedures apply to BSL-2. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted (i.e. doors are closed); and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment. The following special practices, safety equipment, and facility requirements apply to BSL-2:

1. Special Practices
   a. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
   b. Laboratory personnel are offered appropriate immunizations for agents handled or potentially present in the laboratory.
   c. The University and lab specific biosafety manual must be available and accessible.
   d. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
   e. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
   f. Laboratory equipment must be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
   g. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
   h. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
   i. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory PI/supervisor and Biosafety Officer. Animals and plants not associated with the work being performed are not permitted in the laboratory.
   j. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment device.

2. Safety Equipment (Primary Barriers and Personal Protective Equipment)
a. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

(1) Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

(a) High concentrations or large volumes (greater than 10 L as per NIH rDNA Guidelines) of infectious agents are used.

(b) Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

b. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials.

c. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately. Laboratory clothing will not be taken home unless it has been decontaminated. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for splashes or sprays of infectious or other hazardous materials when the microorganisms is handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

d. Gloves must be worn to protect hands from exposure to hazardous materials.

i Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available.

ii Gloves must not be worn outside the laboratory.

iii In addition, BSL-2 laboratory workers should:

(a) Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

(b) Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

(c) Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

e. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

3. Laboratory Facilities (Secondary Barriers)
a. Same as BSL-1 plus the following:
   i  Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
   ii Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
   iii BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
   iv Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps are required.
   v An eyewash station must be readily available.
   vi There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

(1) The Biological Safety Cabinet (BSC) will be tested and certified annually, or after relocation and/or repair, and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified before each use.

(2) A method for decontaminating all laboratory wastes should be available and records maintained in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
Laboratory Biosafety Level Criteria – Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment. A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

1. Special Practices
   a. Same as BSL-2.

2. Safety Equipment (Primary Barriers and Personal Protective Equipment)
   a. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment device.
   b. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls must be worn by workers when in the laboratory. Protective clothing cannot be worn outside of the BSL3. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
   c. Eye and face protection (goggles, mask, face shield or other splatter guard) is required for splashes or sprays, including necropsy activities, of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
   d. Gloves must be worn to protect hands from exposure to hazardous materials.
   e. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available.
   f. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:
      i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
      ii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
   g. Eye, face, and respiratory protection must be used in rooms containing infected animals.
3. Laboratory Facilities (Secondary Barriers)
   a. Laboratory doors must be self-closing and have locks in accordance with
      the institutional policies.
   b. The laboratory must be separated from areas that are open to unrestricted
      traffic flow within the building.
   c. Access to the laboratory is restricted to entry by a series of two self-
      closing doors.
   d. A clothing change room (anteroom) may be included in the passageway
      between the two self-closing doors.
   e. Laboratories must have a sink for hand washing. The sink must be hands-
      free or automatically operated. It should be located near the exit door. If
      the laboratory is segregated into different laboratories, a sink must also be
      available for hand washing in each zone.
   f. Additional sinks may be required as determined by the risk assessment.
   g. The laboratory must be designed so that it can be easily cleaned and
      decontaminated. Carpets and rugs are not permitted. Seams, floors, walls,
      and ceiling surfaces should be sealed. Spaces around doors and ventilation
      openings should be capable of being sealed to facilitate space
      decontamination.
   h. Floors must be slip resistant, impervious to liquids, and resistant to
      chemicals. Consideration should be given to the installation of seamless,
      sealed, resilient or poured floors, with integral cove bases.
   i. Walls should be constructed to produce a sealed smooth finish that can be
      easily cleaned and decontaminated.
   j. Ceilings should be constructed, sealed, and finished in the same general
      manner as walls.
   k. Decontamination of the entire laboratory should be considered when there
      has been gross contamination of the space, significant changes in
      laboratory usage, for major renovations, or maintenance shut downs.
      Selection of the appropriate materials and methods used to decontaminate
      the laboratory must be based on the risk assessment of the biological
      agents in use.
   l. Laboratory furniture must be capable of supporting anticipated loads and
      uses.
   m. Spaces between benches, cabinets, and equipment must be accessible for
      cleaning.
   n. Bench tops must be impervious to water and resistant to heat, organic
      solvents, acids, alkalis, and other chemicals.
   o. Chairs used in laboratory work must be covered with a non-porous
      material that can be easily cleaned and decontaminated with appropriate
      disinfectant.
p. All windows in the laboratory must be sealed.

q. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

r. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps are required.

s. An eyewash station must be readily available in the laboratory.

t. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas.

   1. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

   2. The laboratory exhaust air must not re-circulate to any other area of the building.

   3. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

u. The Biological Safety Cabinet (BSC) will be tested and certified annually, or after relocation and/ or repair, and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified before each use. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs shall be certified annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

v. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

w. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
x. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

y. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing are required to be certified annually.

z. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented annually.

Additional Biosafety Levels
Additional biosafety levels exist for activities requiring specific physical and biological containment practices. These activities are listed below with the appropriate links to requirements:

- **Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses:**

- **Biosafety Levels for Animal Facilities:**

- **Physical and Biological containment for Recombinant DNA research involving animals:**

- **Physical and Biological containment for Recombinant DNA Research Involving Plants (recombinant DNA-containing plants, plant-associated microorganisms, and small animals):**

**SECTION 9 – INCIDENT PREVENTION AND RESPONSE**

**Periodic Review of Risk Assessment Information**
The laboratory supervisor/PI should periodically review information developed from research conducted in the laboratory, as well as that reported by other investigators, that may impact current concepts of risk factors associated with infectious organism.
Incident Investigation

Incidents in laboratories and/or clinics and infections resulting from such work with infectious agents must be promptly reported to the BSO. Prompt and thorough investigations of many of these incidents can identify their causes so that appropriate actions can be taken to prevent similar occurrences.

Reporting of Incidents

All incidents must be reported immediately to the laboratory PI/supervisor and Environmental Health Services (EHS). Such incidents include but are not limited to inadvertent fires, explosions, personnel exposures, injuries, releases of biohazard materials and failure of biological containment. The PI/supervisor must assist EHS personnel with investigations and reports as required. All external reports, other than those of an immediate nature such as summoning the fire department in case of a fire, are to be made by or through the Director of EHS or Biosafety, depending on the incident.

It is important to investigate any serious, unusual, or extended illness of an individual working with biological agents or any accident that involves inoculation of infectious organisms or those containing rDNA molecules.

The investigation of all incidents associated with infectious agents or rDNA research will include a review of techniques, procedures and types and uses of equipment that may have been involved in the accident. The investigation will establish the circumstances leading to the accident. In addition, the investigation report, by the BSO to the Institutional Biosafety Committee (IBC), should provide recommendations for preventing similar occurrences.

All accidents shall be reported as follows:

- Each person involved in biological work shall report to his/her PI/supervisor or EHS:
  - Each incident (both injury causing and those without injury).
  - Each accident resulting in damage to University or other property.
  - Each situation or condition observed on the job which has the potential for either injuring or endangering the health of people and/or causing damage to property or environment.
  - In case of injury, illness, disease, or exposure to infectious material or disease, the person involved or someone on his/her behalf, must report it to EHS within 24 - 48 hours.

- When a person is injured on the job, seek medical treatment as required. (Employees must receive care in accordance with provisions of the University's Worker's Compensation program.) The correct telephone number for emergency medical services shall be posted for ready reference. For campus activities this number is 911.

- Each department is responsible for reporting all accidents to EHS/ Risk Management within four (4) working days. Special reports may be required to properly document the accident for compensation, statistical and accident
prevention purposes. The BSO and/or EHS may be contacted for clarification and assistance in this requirement.

- Serious accidents shall be reported immediately by telephone to CSUPD (911) and to EHS (491-6745). Serious accidents for this purpose are those which result in:
  - Fatality.
  - Hospitalization or medical treatment (beyond first-aid) NOTE: This includes non-CSU personnel.
  - Property damage exceeding $1,000.00.
  - Biological exposure resulting in accidental release of biohazards outside of containment.
  - Infectious Material Incidents (Including rDNA and Infected Animals)

- All incidents involving infectious materials are to be immediately reported to the BSO (or EHS). Such incidents may include spills or releases of materials or agents, escape of infected animals, rupture of plastic bags of infectious/medical waste, or other loss of containment. The BSO or EHS Emergency Responder will direct or oversee cleanup, capture of animals, protection of personnel, and packaging and disposal of the residues.

- Any emergency incident requiring immediate assistance from CSUPD or EHS, or from non-campus agencies such as the fire department, is to be reported immediately to CSUPD Dispatch (911). Such report should tell dispatcher:
  - Where and what type of incident has occurred.
  - Nature and type of any injured or trapped persons.
  - What has happened since the incident: i.e., building evacuation has been started, etc.
  - Identity of caller and location from which he/she is calling and who and where someone will be to meet and/or assist response personnel upon their arrival.
  - Any injury or illness to an employee is to be reported to Risk Management as a Worker's Compensation injury. Employees are to be treated by a designated medical provider; this may be in consultation with the BSO, EHS, PI or supervisor if necessary.

- Students and others not on CSU payroll who are injured or become ill as a result of a biological activity are to be reported. Their medical care is handled separately as dictated by their insurance carrier.

**Recovery After Biological Incidents**

EHS Emergency Coordinators, with assistance from the fire department, State Health, police and/or departments will make determinations that an area/facility/room is safe for re-entry after a biological incident. Others are not to enter or re-enter the area without the consent of the Emergency Coordinator (or Incident Command in coordination with the Emergency Coordinator). The Emergency Coordinator may however, if appropriate,
allow only limited re-entry of specialists who in turn may investigate, remove, rebuild, reinforce, perform temporary fixes or raze the facility as necessary before others are permitted to enter.

**Possible Exposure to Human Body Fluids and Wastes**
Emergency response to accidents, assaults, suicides (or suicide attempts), homicides, etc., where responders could be exposed to potentially infectious body fluids or wastes must be accomplished in a manner that is consistent with the University's Blood borne Pathogen Exposure Control Plan.

**Equipment and Decontamination**
Due to risk of biological exposure, equipment that is or may have been contaminated with biological material, such as a centrifuges, refrigerators, incubators, biological safety cabinets, are required to be decontaminated by the user before it can exit the laboratory to be fixed or sent to surplus. Furthermore, it is recommended that equipment that has contained or may contain biological material be labeled with a biohazard sticker to warn individuals of the biological risk associated with the equipment.

**EMERGENCY CONTACT INFORMATION**
Biosafety related emergency contact information can be found online at [http://www.ehs.colostate.edu/WBiosafety/Home.aspx](http://www.ehs.colostate.edu/WBiosafety/Home.aspx)

Complete CSU emergency contact information can be found at: [www.ehs.colostate.edu/WEmgResp/Home.aspx](http://www.ehs.colostate.edu/WEmgResp/Home.aspx)

Complete list of Current Medical Providers: [http://www.ehs.colostate.edu/WWorkComp/Home.aspx](http://www.ehs.colostate.edu/WWorkComp/Home.aspx)
SECTION 10- EFFECTIVE CHEMICAL DISINFECTION

It is the responsibility of the laboratory PI/ Supervisor to select the appropriate disinfectant for the biological agent(s) used in the laboratory. After selection of a chemical disinfectant effective against the microorganisms being investigated, the laboratory supervisor will need to devise schedules for regular procurement of bulk concentrate and for maintenance of an adequate supply of use concentrations in the laboratory. Follow the manufacturer’s instruction for dilution and use, unless experiments have been performed on the specified organism. Supervisors must devise schedules for disposal of ineffective residual decontaminants and replenishment with fresh solutions. Personal supervision of the application to the spill area of a known effective chemical disinfectant in sufficient concentration with adequate contact time may be the criterion selected by some supervisors for allowing the research to be resumed following the spill.

SECTION 11– SPILL CLEAN UP

The following section describes methods for cleanup of spills under various circumstances.

BSL1 and BSL2 Laboratory Spill Cleanup

Biological Spill Kit Contents

- Gloves; latex and nitrile
- Lab coat; disposable gown
- Face mask or Face shield
- Eye protection
- Booties to protect shoes
- Towels
- Pathogen specific disinfectant
- Brush, dustpan; tongs
- Puncture-proof container for sharps
- Tape
- Marker pen
- Biohazard bags
- Caution sign

Instructions for Personal Protective Equipment (PPE)

- Always use PPE when cleaning up infectious material or when there is the potential for exposure
- Examine PPE to ensure that it is in good condition (damaged PPE must be thrown away)
- Don’t store or stockpile materials for long periods of time
Disposal
- Dispose of all cleanup supplies in the biohazard bag
- Autoclave or contact Environmental Health Services for disposal

Sharps
- Sweep sharp objects, such as broken glass, with a broom and dust pan or use tongs
- Place sharp objects in puncture resistant containers
- Refer to CSU Sharps Policy

Biological Spill Cleanup
- Isolate the area; put up a sign (if available)
- Get the spill kit
- Put on 2 pair disposable gloves, eye protection, gown (if available) and face mask
- Dip towel in disinfectant
- Gently place towels over the spill
- Give the disinfectant time to work (leave 15 minutes or pathogen specific disinfectant contact time)
- Starting from outside of spill area, clean up towels moving inward
- Dispose of towels in biohazard bag
- Repeat process
- Mop surrounding area (10 feet on each side)
- Seal the biohazard bag with tape
- Autoclave or contact EHS for disposal (491-6745)
- If You Are Exposed
  - Cleanse all exposed skin with soap and water for 3-5 minutes
  - Rinse mucous membranes or eyes with water for 15 minutes
  - Record the location and time of incident
  - Report the incident to your PI/supervisor and EHS
  - Seek evaluation at University’s medical provider
  - Fill out an incident report (http://www.ehs.colostate.edu/WBiosafety/Home.aspx) within 24 hours and Worker’s Compensation form (http://www.ehs.colostate.edu/WWorkComp/Home.aspx) within 4 days

BSL3 Laboratory Spills Outside Biological Safety Cabinets
Spills outside biological safety cabinets are complex events. They may involve amounts of material ranging from less than a milliliter up to several hundred milliliters or more. The amount spilled, the physical characteristics of the material, and how the spill occurs are important factors in determining the area of involvement. Each spill is comprised of three somewhat overlapping fractions of the spilled material. The first of these is the bulk of the material that remains in a more or less confluent puddle. The second is that portion separating from the main body of material in large drops and rivulets. The third is that portion that separates from the main body in airborne particulates of various sizes.
The hazard represented by airborne particulates remains largely unknown; however, these small particles have been shown to represent a significant hazard when they contain certain known human pathogens. For some of these, ten or fewer viable particles can cause human infection. The airborne particles emanating from a biological spill are responsible for the preliminary phase of the decontamination procedure. A minimum of 1 hour should be sufficient to achieve a reduction of airborne particles per unit volume permitting the actual decontamination effort to proceed.

**Safety Considerations (Risks)**
There may be unique aspects to a spill incident that are not covered in this manual. In those situations decisions may need to be made in the field that are not specifically stated here.

**Equipment**
- Emergency PAPR

**Materials**
- Large plastic bin - stores all materials
- Small plastic bin - to be used for sharps
- Absorbent materials
- Pathogen specific disinfectant
- Spray bottles
- Gloves (S, M, L & XL)
- Indicator tape
- Autoclave bags (6)
- Marker
- Small dust pan and broom or tongs
- Mop and bucket
- Mop head
- Tyvek suits (L, XL, 2X & 3XL)
- “DO NOT ENTER – Spill Cleanup in Progress” sign (Figure 1.0)
- Locker room sign (Figure 2.0)

**Procedure**
- Exit the spill area. Make sure everyone in the area leaves the area as well.
- Post ‘DO NOT ENTER – Spill Cleanup In Progress’ sign located in the spill kit (figure 1.0).
- If required, Shower Out placing clothes in autoclave trash bin.
- Call Biosafety.
- Wait to be accompanied by Biosafety.
- Wait 1 hour before returning to the spill site.
- You must be accompanied by Biosafety.
- Don regular PPE and Spill Cleanup PPE:
  - Tyvek jumpsuit (Do not put on the tyvek hood)
  - Two pairs of gloves (outer gloves over tyvek sleeves)
  - Emergency PAPR
• Fill mop bucket up with disinfectant per spill kit instructions.
• Soak towels with disinfectant to cover the spill.
• Leave the towels on the spill for 30 minutes.
• Pour more disinfectant as needed to keep the cloth wet.
• Wipe down cabinets, benches, walls, etc. with disinfectant and towels.
• Transfer all materials from the spill cleanup to autoclave bag(s).
• Change outer gloves and seal the bag.
• Mop a 10 ft. radius from the clean side to dirty side of the room, using disinfectant.
• Wipe down cabinets, benches, walls, etc. with disinfectant.
• Remove mop head, place into autoclave bag and wipe down mop handle with disinfectant.
• Remove outer pair of gloves and replace with a fresh pair.
• Remove Tyvek suit with the help of your cleanup partner by turning the suit inside out and discard in an autoclave bag.
• Remove the outer layer of gloves and place into the autoclave bag and put on a second pair of gloves.
• Spray/wipe down all autoclave bags.
• Wipe down PAPR pack and cape hood with disinfectant.
• Place autoclave bags in the autoclave staging area.
• Remove PAPRs outside BSL3 lab and return them to janitor closet.
• Remove ‘Spill Cleanup -In Progress’ sign.
• Shower out.
• File an ‘Incident Report Form’ within 24 hours. Expect to review the incident with your PI/Supervisor, Operations and Biosafety.

Biohazard Spills in Biological Safety Cabinets (BSCs)

Safety Considerations (Risks)
In unique situations, decisions may need to be made in the laboratory that are not specifically stated in this document. A spill that is confined to the interior of the BSC should present little hazard to personnel in the area. However, pathogen specific disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet.

• If a spill exits in the BSC, initiate cleanup of the biological spill outside the BSC first- a biological spill kit should be provided in each area by the PI.
• If chemicals in a lab require special cleanup materials, the lab PI needs to provide the appropriate spill kit from consultation with EHS’-Hazardous Waste group (e.g. acid spill kit).
• If Radiation is involved, contact the EHS Radiation Control Office (491-6745).

Equipment
- N/A

Materials
- Absorbent material (liner, paper towels)
- Pathogen Specific Disinfectant(s)
- Gloves
- Swiffer sweeper or equivalent
- Indicator tape
- Autoclave bags
- Marker
- Liquid waste container (e.g. pipette boat)
- Paper towels

Procedure
If the spill is contained on the liner:
1. Carefully and slowly remove materials away from the spill to the “dirty” side.
2. Materials that are contaminated need to be placed in a liquid waste container or an autoclave bag with disinfectant. They can be retrieved once autoclaved.
3. Slowly fold up the spill in the liner and place gently into the autoclave bag inside the BSC.
4. Remove gloves and put on new pair or if double gloved, remove outer layer of gloves and put on a new layer.
5. Replace materials and resume working in BSC.

If the spill is NOT contained on the liner:
1. Cover the spill with paper towel(s).
2. Replace gloves or replace outer layer of gloves with a new pair if double gloved.
3. Apply the pathogen specific disinfectant to paper towel to disinfect the spill - create a dam around the spill area with the disinfectant if possible to keep the spill from spreading.
4. Allow sufficient amount of contact time (Every disinfectant has an amount of time needed before it is able to kill or prevent the growth of microorganisms - see technical report).
5. Place materials that were splattered by the spill in an autoclave bag or in the pipet boat.
6. Replace (outer) gloves accordingly.
7. Wipe down equipment with a disinfectant saturated towel and let sit for the contact time of the disinfectant if it was involved with the spill area.
8. Replace (outer) gloves accordingly.
9. Thoroughly wipe down the internal surfaces of the cabinet with pathogen specific disinfectant.
10. Place all clean up material into an autoclave bag(s) and seal.
• Replace materials and resume working in the BSC.

If the spill has entered the grill area (KEEP THE CABINET RUNNING):

1. Unscrew the lid of pathogen specific disinfectant and flood the BSC front grille (or back grill if the spill began there) area with the disinfectant.
2. Clean up spill on top (work area) according to Procedure 2. (above).
3. Contact an individual to help with the cleanup process.
4. Using tape, place a new autoclave bag on the inner side of the BSC wall for the biohazardous waste.
5. As a team:
6. Take apart the BSC moving each piece to the inner part of the cabinet and wipe each piece with pathogen specific disinfectant thoroughly before removing the piece from the cabinet.
7. For the work area, (this will take both people) both individuals will hold the work area up and you work together to clean the stainless steel of the underside of the work area.
8. Use a paper towel or swiffer wipe to wipe up the pool of disinfectant under the work area.
9. DO NOT USE YOUR HANDS!
10. Have person not in the BSC assist with providing new paper towels.
11. Replace (outer) gloves accordingly
12. Seal autoclave bag, wipe down and remove from BSC.
13. Place BSC parts back into the BSC accordingly.
SECTION 12 –EQUIPMENT AND LABORATORY PROCEDURES

CSU Sharps Disposal Policy
The CSU Sharps Disposal Policy can be found Under the “CSU Sharps Procedures” tab at:

- http://www.ehs.colostate.edu/WBiosafety/Home.aspx

Engineering controls should be in place when sharps are in use. These include but are not limited to proper sharps containers, blunt ended equipment, broken glass containers and luer lock syringes.

All sharps must be disposed of in the proper container(s) and must be labeled, color coded and properly discarded when the fill line is reached (2/3 full). When sharps containers that have biological materials associated with them are filled to capacity, they must be taped shut, placed into a biohazard bag and properly labeled. Keep all sharps containers upright.

Metal and biohazard sharps:
Any sharps made of metal (including, but not limited to: scalpel blades, razor blades and needles), and/or biohazard-contaminated glassware will be placed in a puncture-resistant container. When said container is 2/3 full, it will be sealed, autoclaved (if necessary), and placed in a cardboard box, which will in turn be sealed. The container and box will both be labeled with the laboratory PI’s name, a contact phone number, the date, and the words, “Decontaminated Laboratory Sharps” written on the top of the box.

To avoid needle stick injuries and possible exposure to hazardous agents, needles should not be recapped, bent, sheared or broken. Retractable needles should be used when appropriate. In rare instances a one-handed technique (eg, use a one-handed scoop technique, or hold the cap with a hemostat or forceps) may be used to recap needles, but this is discouraged. Used needles, as is the case with other sharps, must be appropriately disposed of in a sharps container.

Glassware:
Any non-biohazard-contaminated glassware will be placed in a cardboard or sharps container. When 2/3 full, the box will be sealed and placed in a second box, which will be sealed. The outer box will be labeled with the laboratory PI’s name, a contact phone number, the date, and the words, “Sharps” (and/or) “Broken Glass” written on the top of the box.

EHS does not pick up sharps or glassware. To dispose of the sharps or glassware, check with your particular department/building proctors or custodial staff.

Sharps containers and equipment For BSL3 areas:
- All sharps containers must be plastic, contain ¼ to ½ of pathogen specific disinfectant and be autoclavable.
• The sharps container needs to be in a Biological Safety Cabinet (BSC) or in secondary containment outside of the BSC at all times.
• Leur lock syringes are required to be used when needles with syringes are used or when needles are not permanently attached.

**Biological Safety Cabinets (BSCs), Clean Benches and HEPA-Filtered Exhaust Systems**

Biosafety Cabinet training is required for working in a BSC.

**Types of Biological Safety Cabinets (BSCs)**

• BSCs are classified as Class I, Class II or Class III cabinets. Biosafety cabinets should not be confused with clean benches (or laminar flow hoods) which only provide product protection. Clean benches must never be used with infectious agents.

• Class I BSCs provide personnel and environmental protection, but not product protection.

• Class II BSCs are the most commonly used BSC on the campus. These cabinets provide personnel, environmental and product protection. Only those which are hard ducted to the outside (Class II B2) should be used when working with volatile chemicals. Additionally, personnel using ducted systems must be aware that the cabinets are not designed to prevent ignition of volatile chemicals.

• Class II BSCs come in different types (Type A1, A2 (=A/B3), B1, and B2):
  o Type A1 and A2 exhausts 30% of the air and recirculates 70% through the supply HEPA filter and back into the work zone.
  o Type B1 exhausts 70% of the air and recirculates 30% through the supply HEPA filter, back to the work zone.
  o Type B2 is a total exhaust cabinet, no air is recirculated. This type of cabinet is hard ducted to the outside.

• Class III BSCs are often referred to as glove boxes and provide personnel and environmental protection, but not product protection (no laminar flow). These BSCs have HEPA filter supply air and double HEPA filtered exhaust air and have gloves attached to the BSC. This BSC should be used when generating an increased amount of turbulence and/or aerosol.

**Working in a BSC**

Safety Considerations while working in a BSC

• Be alert, conscientious and pay attention to signs on the BSC.

• Biosafety cabinets should not be placed in high traffic locations or near doors.

• Do not traffic behind BSCs when work is in progress

• Do not block the front or back grille.
All work should be performed ~4 inches from the inside edge of the front grille.

Work following a “clean” to “dirty” flow.

Use a cart or table to stage materials for accessibility for decreased movement when work is in progress inside the BSC.

Minimize the amount of times you pull your hands in and out of the cabinet when work is in progress.

Move arms in and out of the BSC slowly with your arms parallel to the work surface, perpendicular to the sash.

Move in and out of the BSC on your “clean” side.

Only materials and equipment for the specific task should be placed in the BSC.

Use absorbent liner for infectious material work.

Pathogen specific disinfectant and paper towels should be placed on clean side of work area for easy accessibility.

Waste containers (including: sharps containers, autoclave bags and durable containers for liquid and serological pipets) should be placed inside the BSC and should not be taken out of the BSC until properly sealed closed.

Use good microbiological techniques to minimize aerosolization.

The trash cans on the floor beside the BSC are NOT for materials that have come into contact with live organism/ pathogens.

DO NOT use Bunsen burners in BSC.

Serological pipets must be placed in pipet boats (durable containers) filled with pathogen specific disinfectant.

Do not place serological pipets directly into autoclave bags- they will puncture the bag.

Biosafety cabinets should be thoroughly cleaned (under the work area) every one to three months.

Wearing the approved and required Personal Protective Equipment (PPE) will decrease your risk of exposure when working inside the BSC.

Long sleeves with gloves pulled over the sleeves should be used when working in a BSC.

Carefully inspect your gloves for any holes or tears and replace as necessary.

Sharps are not permitted to be disposed of in pipet boats or autoclave bags.
(Refer to CSU Sharps Policy)

How to work in a Class II BSC
The Class II Biological Safety Cabinet can be operated 24 hrs a day. If the cabinet is not left running, the blower or motor should be turned on first, and then the sash should be raised to the correct sash height; wait 5 minutes before work begins to purge any
particulates. When finished using the BSC, wait 5 minutes for the cabinet to purge any residual particulates, close the sash completely and turn off the power to the blower and light.

The Biosafety Office does not advocate the use of Ultra Violet light in the BSC to disinfect the BSC. We recommend using pathogen specific disinfectant to clean the BSC before and after working in the BSC. It is the PI/supervisor’s responsibility to maintain the UV light in the BSC if one is in use.

References
CDC/NIH Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets
CSU Sharps Policy

BSC Certification

Biological Safety Cabinets MUST be certified:
- When they are received. It is extremely important that all new biological safety cabinets be certified when they are received from the manufacturer. Failure to do so may lead to the use of a cabinet which is not functioning appropriately and cause the owner to pay for repairs which should be covered under the purchasing agreement.
- When they are moved.
- Annually.

Certification of new cabinet

Provide the Department or contractor with the following information:
- The name of the principal investigator
- Department in which the cabinet belongs
- Manufacturer’s name, model number, serial number
- Location of the cabinet (building and room number)

The Department will provide you with the name and phone number of the company which has contracted with the University to certify cabinets. Most cabinets on campus are certified during an annual certification period. At that time, a designated contact person for your department will be given a list of cabinets and asked if you would like to have the cabinet certified. The Biosafety Cabinet certifying agent will be contacted to provide certification to all BSC’s that need certification (A new BSC, annual certification, or a BSC has been moved). It is the responsibility of the PI to ensure that his/her cabinet is certified on an annual basis and to bear the cost of the certification.
Certification Contract
CSU maintains a certification contract with a private company to provide for (1) certification and testing of laminar flow clean benches/ hoods, biological safety cabinets, animal cages and HEPA filtered exhaust systems and (2) the repair of the above mentioned equipment. Problems with the contractor's work should be referred to the Biosafety Officer or Purchasing Agent in charge of the contract.

Autoclave and Steam Sterilizer Inspection, Maintenance and Certifications

Autoclaves and other steam sterilizers are important decontamination systems used in research with potentially hazardous microorganisms. They are used as the principal devices for sterilizing contaminated wastes to insure safe disposal. (Ethylene Oxide sterilizers may also be used in certain applications where items to be sterilized may be adversely affected by steam sterilization conditions.) Good safety management requires that the efficacy of these sterilization devices be verified before and during usage for sterilization of materials contaminated with potentially hazardous microorganisms.

Autoclaves and steam sterilizers are pressure vessels requiring periodic testing and maintenance to assure their operability and safety. Facilities Management (or a contractor arranged through and with the concurrence of Facilities Services) must perform periodic testing and maintenance of these units.

Users of autoclaves and steam sterilizers need to be trained on how to use the autoclave correctly and shall use chemical/ temperature indicators with each load of material to be sterilized. Biological indicator validation tests should be performed at least every 6 months for all autoclaves. Autoclaves in BSL-3 areas should use biological indicators weekly. Any deficiency noted during these tests or with the other indicators, visually or as a result of recorded temperatures, is cause for stopping the use of the unit until repairs and recertification are completed and documented.

Autoclaves and steam sterilizers are to be inspected and maintained in accordance with manufacturer's recommendations and as additionally specified herein. The department is responsible for repair and maintenance of the autoclaves.

Autoclaves and steam sterilizers which are found to be satisfactory after maintenance testing shall be "certified" for use by the maintenance technician performing the test. The technician (whether Facilities Services or contractor) must be trained in maintenance and testing to the satisfaction of the BSO and/or Director of Environmental Health Services (EHS). "Certified" units will be individually marked, as a minimum, to show certification, date next testing is due and individual performing certification test. Units with overdue certification testing should be marked to show that they are not certified and are possibly unsafe. Unsafe units will be tagged "Danger - Do Not Use" until repairs are completed and the unit is recertified.
Protection of Vacuum Systems from Biological Agents

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure. To prevent the accidental contamination of house vacuum system or vacuum pumps, protection should be provided against pulling biohazardous aerosols or overflow fluid into the vacuum system. This protection is provided by the use of an air filter in the line immediately leading into the house vacuum line and an overflow flask for liquids between the collection flask and the air filter.

Techniques for protecting the vacuum system are described as follows:

A cartridge-type filter provides an effective barrier to passage of aerosols into the house vacuum system. The filter has a capacity to remove airborne particles 450 nm (0.45 μ) or larger in size. (Ultipor, DFA 3001 AXPK5, from the Pall Corporation, Courtland, New York 13045 is an example of such a filter.) However, one must be aware of the size of organism that is aerosolized while using this system.

Flexible tubing is used with an appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flasks of capacities from 250 to 4000 ml may be used for the overflow flask, depending on available space and amount of fluid that could be accidentally aspirated out of the collection flask.

NOTE: Appropriate safety precautions (taping of flasks, secondary containers, eye protection, etc.) are required during installation, operation and servicing of this equipment.

The overflow flasks contain a disinfectant solution appropriate for the biohazardous material under study. It is essential that an anti-foam, such as DOW Corning Antifoam A, be added to the overflow flask, since bubbling of air through the disinfectant probably will cause considerable foam which, if allowed to reach the filter, will shut off the vacuum.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter can then be installed and the assembly replaced.

One type of apparatus can be composed of two suction flasks, a filter, rubber stoppers, flexible vacuum tubing, glass tubing, and a small glass sparger. Various small fitted glass or ceramic spargers or gas dispersion tubes are commercially available. The coarse or medium porosity sparger assures that any aerosol passing through the collection flask is dispersed in small bubbles so that adequate contact is made with the disinfectant solutions.

Another apparatus that can be used has the feature of automatically shutting off the vacuum when the storage flask is full. It consists of a 1 L filter flask with a small glass Buchner funnel (15 ml capacity, 29 mm filter disk) inserted upside down in a No. 8 rubber stopper in the mouth of the flask. A hole, 2 cm in diameter, is cut into the bottom of the stopper with a cork borer and of sufficient depth that the filter disc is level with the
bottom of the stopper. A 1/2 oz rubber bulb measuring 2 3/8 inches in length and 1 1/4 inches in diameter, with the end plugged with a solid glass rod measuring 1/4 inch in diameter and approximately 2 1/2 inches in length, is placed inside the flask.

If liquids enter the overflow flask, the rubber bulb rises until it presses against the mouth of the Buchner funnel and shuts off the vacuum. The entire unit is autoclavable, but the filter assembly should be thoroughly dried before reuse. A commercial version of this apparatus is available. (Vacuum Guard II, Model VG 201, Spectroderm International, Inc., Fairfax, VA 22030).

Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to inactivate the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

SECTION 13 –TRANSPORTATION AND SHIPPING

Transportation of Materials
Protective secondary containers for transporting potentially infectious materials are effective in preventing spills. The use of secondary protective containers is strongly recommended whenever transporting liquid material and is mandatory for transit of infectious materials within the corridors serving the laboratories.

IATA and DOT Regulations for Transport, Shipping and Receiving Biological agents
The IATA Dangerous Goods Regulation (DGR) is the industry standard for transporting dangerous goods by air. While IATA is not a federal or international regulatory agency, in general, unless the IATA DGR is followed for the air transport of dangerous goods, air carriers will not accept the shipment. IATA does not apply to packages that are shipped exclusively by ground transportation.

DOT regulates the transport of hazardous materials to, from or through the United States. DOT regulations are found in part 49 of the Code of Federal Regulations (49 CFR), are enforceable by law, and can carry significant fines and other penalties for failure to comply. These regulations and requirements apply to anyone who, with respect to dangerous goods or hazardous materials:
Handles
- Offers for transport
- Transports
- Causes dangerous goods to be transported
- Loads/unloads transport vehicles or aircraft
- Determines the hazard class of a hazardous material
- Selects hazardous materials packaging
- Fills a hazardous materials packaging
- Secures a closure on a filled or partially filled hazardous materials package
- Marks a package to indicate that it contains a hazardous material
- Labels a package to indicate that it contains a hazardous material
- Prepares a shipping paper
- Provides and maintains emergency response information
- Reviews a shipping paper to verify compliance with the Hazardous Materials Regulations or international equivalents
- Manufactures and/or tests packaging materials for dangerous goods use.

Training Requirements
Both DOT and IATA have specific training requirements for persons who package and ship certain hazardous materials. Among these hazardous materials are infectious (etiologic) substances, diagnostic specimens, biological products, genetically Modified Organisms, or genetically modified microorganisms (GMOs and GMMs).

DOT requires initial training for anyone who prepares packages for shipment which includes general awareness/familiarization, function-specific, and safety training. In addition, Security Awareness Training is mandatory which provides an awareness of security risks associated with hazardous materials transportation, methods designed to enhance transportation security and how to recognize and respond to possible security threats. Recurrent training is required every three years under the DOT regulations.
IATA requires similar training, but recurrent training is required every two years OR as often as the regulations change, which tends to occur annually.

Refer to: General Concepts and requirements section: Dangerous Goods 6.2 (Infectious Substance Shipping, Transporting, and Receiving) and Dangerous Goods 9 Misc. (Dry ice and Genetically Modified Organisms/ Micro-organisms)

Permits

Select Agent
The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules for the possession, use, and transfer of select agents and toxins (42
C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) in the Federal Register on March 18, 2005. All provisions of these final rules superseded those contained in the interim final rules and became effective on April 18, 2005.

**Ship Select Agent from Laboratory**

IATA/DOT Dangerous Goods 6.2 training certification and Security Risk Assessment (SRA) approval is required for shipping or receiving Select Agents internally or externally.

The PI and Biosafety Officer are responsible to complete the required documentation before preparing the package for shipment. The PI is responsible to supply:

- Required permits
- Pertinent information for the Biosafety Officer to fill out APHIS/CDC Form 2.
- Biosafety will fill out APHIS/CDC Form 2 Section 1B, 1C, Section 2.

Once the shipping transfer documentation is approved by the APHIS/CDC/USDA:

- Record the shipment of the select agent(s) in the inventory log.
- Triple pack samples using the correct packaging materials as referred to in IATA/DOT training.
- Liberally apply disinfectant to wipe down between each pack and keep visual contact of the triple packed sample(s) at all times.
- Shower out of the barrier and dress in the locker room with the packed sample(s) OR place the triple packed sample(s) on the material transfer cart and have another SRA approved individual take the package from there to continue with the shipping process. (Refer to Material Policy).
- Package according to IATA and DOT standards (refer to the current IATA manual) and include correct shipping declarations and other documentation (permits) inside and outside the package accordingly.
- Have Biosafety fax APHIS/CDC Form 2 to the CDC then place a copy of APHIS/CDC Form 2 in the box.
- Biosafety, or SRA approved certified shipper, will contact Central Receiving (491-2742/ 491-5347) before product enters the Central Receiving facility to insure a designated select agent approved individual can take the package and secure it until pickup by carrier.
- Biosafety, or SRA approved certified shipper, will drive the package to central receiving.
- A designated SRA approved individual will be present to receive the package, inspect it and place it in the lock cage until it is picked up by a certified carrier.
- Bring picture identification.
- Shipping certification will be verified when presenting shipment.
Email the tracking number of the shipment and the expected arrival time to all corresponding parties. (Biosafety officers from both the outgoing and receiving facilities, and any other individual(s) involved in the shipment.)

Receive Select Agent into Laboratory:
IATA/DOT certification and Security Risk Assessment (SRA) approval is required for persons that will be shipping or receiving Select Agents internally or externally.

PI and Biosafety officer fill out required documentation for the package before the package is shipped to CSU. The PI is responsible to supply:

- Required permits
- Pertinent information for the Biosafety Officer to fill out APHIS/CDC Form 2.
- Biosafety will fill out APHIS/CDC Form 2 Section 1A (See unit 6 and 7 training materials).
- The package will be delivered to Central Receiving by a certified carrier.
- A Biosafety officer or SRA approved certified receiver will pick up the package from Central Receiving.
- Bring picture identification
- Shipping certification will be verified before shipment can be picked up.
- Central Receiving will have a SRA approved certified receiver retrieve the package from the locked cage.
- A Biosafety officer or SRA approved certified receiver, will drive the package to an approved Select Agent facility, and deliver it to the SRA approved recipient lab.
- Paperwork is removed from the box in a BSL2 lab.
- For BSL3 Select Agents the triple packed container is:
  - Delivered to the BSL3 lab
  - Checked for accuracy in the Biological Safety Cabinet (BSC)
  - Added to the inventory.
- For BSL2 Select Agents the triple packed container is:
  - Checked for accuracy in the Biological Safety Cabinet (BSC) and
  - Added to the inventory.
- APHIS/CDC Form 2 Section 3 is then completed and faxed to the CDC and to the shipping entity by the Biosafety officer.
- A confirmation of receipt by the CDC should then be received and the shipment is closed.
Leaking package

- The package is immediately placed in a plastic garbage or biohazard bag and sealed, while using proper PPE to handle the package (gloves, N95 or something to cover the face). Treat area as a spill outside the Biological Safety Cabinet (BSC).

- Biosafety Officer is immediately contacted.

- The bag with the leaking package is placed in a certified BSC to determine where the leak is coming from (e.g. from freezer packs, water from an outside source, infectious substance).

- If leak is from infectious substance, the Biosafety Officer immediately contacts the CDC and an APHIS/CDC Form 3 is filled out.

Manual References


N.I.H. Laboratory Safety Monograph, a supplement to the NIH "Guidelines for Recombinant DNA Research", Jan. 1979
APPENDIX 1 – EXAMPLES OF ORGANISM RISK GROUPS

Examples of Risk Group 1 Agents:
- *Escherichia coli*-K12
- *Bacillus subtilis* or *Bacillus licheniformis*
- Adeno-associated virus types 1 through 4

Examples of Risk Group 2 Agents:
- *Borrelia burgdorferi*
- *Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen
- *Mycobacterium* (except those listed in Risk Group 3) including *M. avium* complex
- *Staphylococcus aureus*
- *Salmonella enterica*
- *Leishmania* including *L. major* and *L. mexicana*
- *Toxoplasma* including *T. gondii*
- Adenoviruses, human - all types
- Eastern and western equine encephalomyelitis virus
- Yellow fever virus vaccine strain 17D
- Rabies virus - all strains

Examples of Risk Group 3 Agents:
- *Brucella* species
- *Mycobacterium bovis* (except BCG strain)
- *Mycobacterium tuberculosis*
- *Rickettsia* species
- *Yersinia pestis*
- *Histoplasma capsulatum*
- Venezuelan equine encephalomyelitis virus (except vaccine strain TC-83 - RG2)
- Japanese encephalitis virus
- Human immunodeficiency virus (HIV) types 1 and 2

Examples of Risk Group 4 Agents:
- Lassa virus
- Crimean-Congo hemorrhagic fever virus
- Ebola virus
- Herpesvirus simiae (Herpes B or Monkey B 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 and they are commonly used in laboratory experimental work. For those agents that do not infect human cells, a containment level appropriate for Risk Group 1 human agents is
recommended. A containment level appropriate for Risk Group 2 human agents is recommended for those that do infect human cells.

- Baculoviruses

- Herpesviruses (H. ateles, H. saimiri, Marek's disease virus, murine cytomegalovirus)

- Papovaviruses (Bovine papilloma virus, Polyoma virus, Simian virus 40)

- Retroviruses (Avian leukosis virus, Bovine leukemia virus, Feline leukemia virus, Feline sarcoma virus, Gibbon ape leukemia virus, Mason-Pfizer monkey virus, Murine leukemia virus, Murine sarcoma virus)

**Virus Vectors**

Murine retroviral vectors to be used for gene 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.

Biosafety level Criteria

Refer to: Biosafety in Microbiological and Biomedical Laboratories: Biosafety Guidelines and Biosafety Levels (BSL1-4).