Contents
SECTION 1 - Introduction .................................................................................................................. 4
SECTION 3 - General Biosafety ........................................................................................................ 13
SECTION 4 – Recombinant and Synthetic Nucleic Acid Research ................................................... 19
SECTION 5- Training and Enrollment Requirements ........................................................................... 20
  Institutional Biosafety Committee Training .................................................................................. 20
  Online Biosafety Level (BSL) 1 and BSL 2 Training ................................................................. 20
  Biological Safety Cabinet (BSC) Training ..................................................................................... 20
  Blood Borne Pathogen (BBP) Training (Human Samples) .......................................................... 20
  Dangerous Goods 6.2 (Infectious Substance Shipping, Transporting, Receiving) and Dangerous Goods 9 Misc. (Genetically Modified Organisms/ Microorganisms and Dry Ice) ................................................................. 21
  Facility Orientation Training/ Tour ............................................................................................... 21
  Mock BSL3/Animal BSL3 (ABSL3)/ Arthropod Containment Level 3 (ACL3) Laboratory and Biosafety Cabinet Training ................................................................. 21
  General characteristics and clinical symptoms associated with infectious agents in the BSL3/ABSL3/ ACL3 laboratory .................................................................................................................... 22
  Barrier training inside the BSL3/ ABSL3/ ACL3 with a lab specific trainer ............................. 22
  Tier 1 and Select Agent and Toxin Regulations ............................................................................. 22
SECTION 6- Occupational Health Program ....................................................................................... 23
SECTION 7 - Risk Assessment and Risk Groups ................................................................................ 23
SECTION 8 - Biosafety Guidelines and Biosafety Levels .................................................................. 27
  Standard Microbiological Practices to be followed at All Biosafety Levels .............................. 27
  Special Practices, Safety Equipment, and Laboratory Facility Requirements ......................... 29
    CSU Undergraduate Labs ........................................................................................................... 29
  Biosafety Level 1 .......................................................................................................................... 29
  Laboratory Biosafety Level Criteria – Biosafety Level 2 .......................................................... 31
  Laboratory Biosafety Level Criteria – Biosafety Level 3 .......................................................... 34
  Additional Biosafety Levels ......................................................................................................... 39
SECTION 9 – Equipment and Laboratory Procedures ....................................................................... 39
  Biological Safety Cabinets (BSCs), Clean Benches and HEPA-Filtered Exhaust Systems .......... 39
  CSU Sharps Policy ....................................................................................................................... 43
SECTION 10- Biological Decontamination ......................................................................................... 50
SECTION 11- Incident Prevention and Response ............................................................................ 53
EMERGENCY CONTACT INFORMATION.................................................................57
SECTION 12– Spill Clean Up ..................................................................................58
   BSL1 and BSL2 Laboratory Spill Cleanup ..........................................................58
   BSL3 Laboratory Spills Outside Biological Safety Cabinets ..................................59
   Biohazard Spills in Biological Safety Cabinets (BSCs) ...........................................61
SECTION 13 –Transportation and Shipping ...............................................................63
Transportation of Materials ....................................................................................63
Manual References ..................................................................................................66
APPENDIX 1 ............................................................................................................66
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 or synthetic nucleic acid molecules.

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 in Microbiological and Biomedical Laboratories (BMBL): [http://www.cdc.gov/biosafety/publications/bmbl5/](http://www.cdc.gov/biosafety/publications/bmbl5/) (the latest edition of the BMBL is to be used as a reference).

As used herein, the words "must," "will" or "shall" indicate mandatory requirements whereas the words "may", "should" or "recommend" indicate items for consideration as “best 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](http://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 biological hazards will follow this manual 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 / Responsible Official (RO) may 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 manual. However, where the provisions of this manual 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 persons 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 the IBC.

**Institutional Biosafety Committee (IBC)**
The IBC at CSU provides local review and oversight of nearly all forms of research utilizing biohazardous materials and recombinant or synthetic nucleic acid molecules. The IBC ensures that recombinant and synthetic nucleic acid research conducted at or sponsored by CSU is in compliance with the *NIH Guidelines*. The IBC adopts procedures and controls specified herein with the advice and consent of the University's Vice President for Research (VPR) and the 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 the supplements 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 or synthetic nucleic acid molecules depends on the individual conducting such activities. It is not possible to anticipate every possible situation. 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 (Biosafety) Officer(s), 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 or synthetic nucleic acid molecules 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; grants, 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, Vice President For University Operations, Responsible Official (RO) and Alternate Responsible Officials (ARO), Biosafety Officers, Environmental Health Services, Deans, Department Chairs, Principal Investigators, Supervisors, and the Individual Employee.

Additionally, each institution conducting or sponsoring recombinant or synthetic nucleic acid molecule 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 and synthetic nucleic acid 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.
  - NIH mandated IBC Requirements:
  - 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 or synthetic nucleic acid molecules, infectious biological material, biological toxins, animal models, plant models and biological safety.

Responsibility
The IBC is responsible for the review, approval, and oversight of all biological agents and research projects involving potentially biologically hazardous and recombinant or synthetic nucleic acid 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:
- [https://www.research.colostate.edu/orcc/ibc/](https://www.research.colostate.edu/orcc/ibc/)

Biological Safety Officers (BSOs)
CSU has one Biosafety Office Director, and one or more Associate or Assistant Biosafety Officers. The Biological Safety Officer shall be a voting member of the IBC. 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;
- Plan and develop policies and procedures that comply with federal select agent regulations promulgated under the authority of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002.
- Ensure that CSU is in compliance with the select agent regulations and serves as the main point of contact for all select agent registration, reporting, and compliance issues.
  - The regulatory requirements for the RO and ARO are found in Section 9 of the select agent regulations.
    - The latest electronic version of this document can be found at [http://www.selectagents.gov](http://www.selectagents.gov)
• Reporting to the IBC and the institution any significant problems, violations of the NIH and BMBL Guidelines, local or federal laws, or other CSU established policies, and any significant research-related incidents or accidents of which the BSO(s) become aware;

• Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory incidents involving biological and recombinant or synthetic nucleic acid research;

• Providing advice and developing plans on laboratory security;

• Providing technical advice to Principal Investigators and the IBC on research safety procedures;

• Developing and conducting appropriate training programs and standard operating procedures for safe handling, transport, and disposal of biological hazards;

• Investigating incidents 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:

• Obtaining IBC approval prior to initiating or modifying work with potentially biohazardous materials, rDNA or synthetic nucleic acid molecule research.
  
  o Make an initial risk assessment in order to determine the required levels of physical and biological containment in accordance with this manual, CSU policies and procedures, Select Agent Program (if select agents will be used) and NIH Guidelines (if recombinant or synthetic nucleic acids will be used);
  
  o Select the appropriate microbiological practices and laboratory techniques to be used for the research, as appropriate to the organism and the research being performed;
  
  o Ensuring IBC forms are accurate and up to date by submitting annual renewals and amendment requests prior to initiating any changes.
• Promptly reporting any significant problems and/or violations of the NIH Guidelines, BMBL, Select Agent Program, Federal and State laws, or CSU policies/procedures, or any significant research-related incidents and accidents to the BSOs (Responsible Official (RO)/ Alternate Responsible Officials AROs)) and the IBC, and also to the following, where applicable: Building Directors/ Proctors, Greenhouse or Animal Facility Directors, EHS and other appropriate authorities (if applicable). The BSO (RO/ AROs) will report to the CDC/ USDA and/or FDA and the IBC Coordinator will report to the NIH Office of Science Policy (OSP) as applicable.
  - Immediately reporting any incident or accident involving biological material (including but not limited to: spills, an exposure involving recombinant or synthetic nucleic acid molecules research, or an exposure that otherwise could lead to personal injury or illness, or to a breach of containment).
    https://www.research.colostate.edu/orcc/ibc/reporting-a-biosafety-concern/

• Being familiar with and complying with the United States Government (UGS) and CSU Dual Use Research of Concern (DURC) Policies.
  - Dual Use Research of Concern (DURC) is defined as: life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. The United States Government’s (USG) oversight of DURC is aimed at preserving the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research. Although few research activities at CSU would qualify as DURC under the definition in the Policy, CSU must comply with the USG Policy for Institutional Oversight of DURC. For more information regarding the scope of this policy, and the roles and responsibilities of the institution and the PI, please see the CSU’s DURC policy.

• Adherence to approved emergency plans for handling spills and personnel contamination;
• Compliance with shipping requirements for biological materials and recombinant or synthetic nucleic acid molecules;
  o Refer to IATA, DOT Dangerous Goods Regulations and Appendix H in the NIH Guidelines.

**Responsibilities of the PI Prior to Initiating and during the Conduct of Research**

The Principal Investigator shall:

• Be adequately trained in good microbiological techniques; and ensuring that all personnel are appropriately trained.
  o Make available to all laboratory staff the protocols that describe the procedure, potential biohazards and the precautions to be taken;
  o Assure appropriate instruction and training of staff, including but not limited to: (i) required institutional training modules; (ii) the risks associated with the work being performed and how risks are mitigated; (iii) the practices and techniques required to ensure safety, and (iv) the procedures for dealing with incidents and accidents;
  o Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations, medical evaluations/clearance, serum collection).

• Ensure that the required safety practices, techniques and personal protective equipment (PPE) are provided and employed;

• Identify and correct work errors and conditions that may result in the release and/or exposure of biological, recombinant or synthetic nucleic acid materials;

• Ensure the integrity of physical containment (e.g., biological safety cabinets, autoclaves) and biological containment (e.g., purity and genotypic and phenotypic characteristics).

• Adhere to the BSL audit requirements outlined in the policy: [https://drive.google.com/file/d/0B4czSBPPYW40c21wb2thOFlkU0E/view](https://drive.google.com/file/d/0B4czSBPPYW40c21wb2thOFlkU0E/view)

**Responsibilities of the PI prior to leaving the University or moving lab space**

• Notifying the IBC and BSO prior to leaving, transferring agents/projects to another PI, or moving lab space.
o Close out AARF(s) and/or PARF(s)
o Appropriately transfer AARF(s) and/or PARF(s) to a different PI with the aid of the IBC coordinator.

- Complete a final lab audit and remove all biological material appropriately

- Decontamination of equipment and lab space.
  o Clean out and decontaminate materials in the cold/ warm room, storage areas and common equipment areas as well.

For information regarding the roles and responsibilities of NIH, OSP and the Recombinant DNA Advisory Committee (RAC) please refer to the following website: https://osp.od.nih.gov/biotechnology/nih-guidelines/.

**Each Individual**

Personnel are responsible for ensuring safe practices and compliance during the conduct of research. The Individual performing the research shall:

- Adhere to CSU policies and guidelines outlined in this manual and in their applicable Lab Specific Biosafety Manuals. Since not all circumstances can be foreseen or described in this manual, it is the responsibility of the individual to use good judgment and to adhere to the INTENT of the guidelines as well as the specifics.

- Immediately report all research related illnesses, accidents, incidents, and/ or violations to the PI, supervisor, BSO, IBC Coordinator, Occupational Health Coordinator and Greenhouse/Animal Facility Director (where applicable).

- Be familiar with and know the hazards of the materials or substances used.

- Be familiar with and know the guidelines, rules and/ or regulations pertaining to the activities performed.

- Carefully observe proper procedures and protocols.

- Have appropriate training as required by Federal, State, and CSU requirements and consult with PI/ supervisors to receive appropriate instruction and training specific to the lab, such as the handling and disposal of biohazardous materials utilized in the workplace and incident management procedures.

- Correct any unsafe conditions or practices observed and/or report any unsafe conditions or practices to the responsible PI, supervisor, and the BSOs.

- Pregnant women and persons who are immune-compromised or have other health conditions are encouraged to consult with their supervisor, Occupational
Health Services, BSOs or physician of choice concerning potential risks and management of these risks.

SECTION 3 - General Biosafety

The CSU community complies with the biosafety program to provide safety for the individual performing the research with biological material. The Biosafety Officers advise and assist with 1) Engineering and Maintenance 2) Facility Plans and Workplace Practices and 3) Compliance, Education and Training.

1) Engineering and Maintenance
Engineering and maintenance consider both the workplace conditions as well as the people working in those conditions and includes: a) the design, construction, renovation/ modification, certification and maintenance of the laboratory; b) the selection and monitoring of laboratory equipment; c) the destruction or safe disposal of biological waste; and d) the decontamination of lab areas and equipment no longer to be used in biological activities.

a) Design/ Construction/ Renovation/ Modification/ Certification/ Maintenance
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 or Synthetic Nucleic Acid Research (NIH Guidelines) and other criteria specified by the Federal Government and the Biosafety Office/ IBC in addition to requirements of the University's Building and Fire Code. The most current edition of these guidelines will be used. The IBC will approve such design and the BSO shall review the design and construction as well as observe the final completion of the work before biological projects are started in new or remodeled facilities.

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.

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.

b) Selection and Monitoring of Laboratory Equipment
The BSOs/IBC shall approve operation of any equipment that may generate aerosols or pose other biosafety concerns.

c) Decontamination, Destruction and Safe Disposal of Biological Waste
There are multiple avenues of decontamination, destruction and safe disposal of biological waste. The BSOs will assist in the selection of the appropriate method and the IBC will approve the method selected. Refer to Section 10 of this manual.

2) Facility Plans and Workplace Practices
Emergency Action Plans for individual facilities should be made available to employees and to emergency response personnel so they can better respond to 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, the PI’s delegate, 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. Safety Data Sheets (SDSs) or similar information regarding biological materials or substances involved or possibly encountered must be made available to personnel.

PIs and others proposing to work with biological materials must develop detailed procedures and safe work practices for those activities and see that they are followed when approved. Whether on paper or electronically, these procedures and safe work practices need to be centrally located in a lab specific biosafety manual, in accordance with the BMBL and NIH Guidelines, to assure ready availability and accessibility to all employees. Construction of the lab specific biosafety manual is facilitated by the IBC and the Biosafety Office.

In order to establish that each employee has been specifically trained and is aware of the critical biosafety information outlined in the lab specific biosafety manual, it is important to maintain signed acknowledgement for each employee that they have read and understood the information provided in the document and intend to comply.

When required by directives or when required by this manual, these procedures, including the safe work practices, special work practices and instructions on how to use lab specific equipment safely are to be submitted to the IBC and/or the BSOs. The IBC and/or BSOs shall review hazards and procedure(s) involved and, as appropriate, approve, direct or suggest changes.

3) Compliance, Education and Training
All persons involved in biological activities, whether faculty, staff, visitors 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 Policy on Biosafety and Biohazards non-compliance has been posted on the ORCC/IBC website under policies: https://www.research.colostate.edu/orcc/ibc/policies-and-regulations/

The BSOs/IBC review all biological activities through inspections/audits of both work practices and the work environment.

a) Lab Audits

i) The CSU Biosafety Office will perform BSL1 laboratory audits for a new lab or for a BSL1 lab that has not been audited before. It is then the responsibility of the BSL1 lab to complete a self-audit yearly after that initial lab audit and will be asked by the Biosafety office for the completed lab audit documentation.

ii) The CSU Biosafety Office will perform BSL2 lab audits for a new BSL2 lab or for a BSL2 lab that has not been audited before. The BSL2 lab is then responsible to self-audit for the next second and third years. One of the biosafety officers will audit the BSL2 labs every three years, or as otherwise required, to ensure compliance with the procedures and protocols of this manual and the lab’s specific biosafety manual.

iii) Each PI/supervisor is required to do self-audits annually and send the report to a biosafety officer annually. Audit reports will document compliance and noncompliance with approved laboratory protocols and be directed to the PI or their delegate. The results of these audits will be documented and kept on file with the Biosafety Office.

iv) Corrective actions, to the satisfaction of the IBC, are to be taken for each deficiency noted. Any significant problems will be reported to the IBC.

v) The IBC, with the assistance of the biosafety officers, will oversee and ensure compliance with the provisions of individual project protocols, legal requirements, this manual and University Policy.

vi) 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 BSOs, the IBC and EHS if needed.
vii) Action items on the audits need to be taken within 1 month of the audit, or sooner for items that pose imminent threat to safety.

b) Requirements for Biological Laboratories

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

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

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

iv) Other

(1) Diagnostic, Analytical and Clinical Laboratories. These laboratories 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.).

a) 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 that address the issue of the potentially infectious hazard of specimens.

b) 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 Biosafety Level 2 (BSL2). Biosafety Cabinets (BSCs) (Class I or II) shall 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. Class II type B2 cabinets need to be used when
hazardous chemical and hazardous biological work will need to be performed. 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).

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

(2) Field Studies. Biological field studies are studies that occur in the natural environment. Personnel should consult with the Biosafety Office for a risk assessment prior to conducting a biological field study. Biological 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 the Environmental Protection Agency (EPA).

(a) 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 approval by the IBC.

c) Education and Training

All persons should meet the minimum requirements, as indicated by the BSOs and guidelines issued by the BMBL and NIH Guidelines for the area or activity involved. Education requirements are developed by the Biosafety Office with approval of the IBC. Individuals directly involved with biological agents must be formally trained for their specific tasks by the PI (or his/ her delegate), the Biosafety Office and other pertinent organizations (LAR, EHS, IACUC).

Lab specific training is provided by the PI/ Supervisor. The PI/ Supervisor may assign a lab manager or specific trainer to train an individual; however, the PI/ Supervisor 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, including emergency procedures.

All persons working with or around biological agents must:
Be instructed in standard and special microbiological practices associated with their Biosafety Level (BSL), entry and exit control procedures; equipment used during manipulation of the agent; the meanings of the various signs, controls and lab procedures used; emergency procedures applicable 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:

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 Office with support by the IBC in accordance with the potential exposure.

Training certifications/documentations will be maintained as required by the PI/Supervisor and the Biosafety Office and additional education or training requirements are to be imposed as stated. Refer to Section 4 of this manual.

d) Requirements for Visitors and Untrained Personnel

i) 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, unless approved by the BSO/IBC. This restriction is to protect the individual, equipment, supplies, work in progress and experimental animals or plants. Minimum requirements are specified by the type of activity within individual chapters of this manual. (See Sections 4, 5, and 7)

ii) 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.
iii) Copies of Safety Data Sheets (SDS), when requested, shall be provided to any emergency response personnel by the PI/supervisor.

iv) 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.

SECTION 4 – Recombinant and Synthetic Nucleic Acid Research

As a term and condition for NIH funding for research involving recombinant or synthetic nucleic acid molecules, CSU must ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, complies with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). CSU receives NIH funding for research involving recombinant or synthetic nucleic acid molecules, therefore ALL research at CSU involving recombinant or synthetic nucleic acid molecules must comply with the NIH Guidelines.

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) detail safety practices and containment procedures for basic and clinical research involving recombinant or synthetic nucleic acid molecules, including the creation and use of organisms and viruses containing recombinant or synthetic nucleic acid molecules.

The purpose of the NIH Guidelines is to specify the practices for constructing and handling: (i) recombinant nucleic acid molecules, (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (iii) cells, organisms, and viruses containing such molecules.

The NIH Guidelines require that CSU reports any significant problems, violations, or any significant research-related accidents and illnesses to the Office of Science Policy (OSP) NIH within 30 days. Therefore it is imperative that any and all laboratory incidents be reported immediately (within 24 hrs.) to the Biosafety Office. Please see the IBC Policy on Reporting Incidents Involving rDNA and synthetic nucleic acids: https://www.research.colostate.edu/orcc/ibc/policies-and-regulations/

See https://osp.od.nih.gov/biotechnology/nih-guidelines/ for the complete NIH Guidelines required for CSU recombinant and synthetic nucleic acid research.
SECTION 5- Training and Enrollment Requirements

CSU Biosafety training addresses a multitude of requirements established by the 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: [http://www.ehs.colostate.edu/WTrainReg/ClassSignUp.aspx](http://www.ehs.colostate.edu/WTrainReg/ClassSignUp.aspx)

**Institutional Biosafety Committee and Principal Investigator Training**
This training is required every three years for all individuals submitting agent and project approvals to the IBC. This training covers the NIH recombinant and synthetic nucleic acid molecule guidelines.

**Online Biosafety Level (BSL) 1 and BSL 2 Training**
This training outlines the requirements and safety practices for working in BSL1 and/or BSL2 laboratories. Training is required every 2 years unless the individual has completed the BSL1,2,3 Concepts Training, in which case, training is only required once.

**Biological Safety Cabinet (BSC) Training**
This 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. (Genetically Modified Organisms/ Micro-organisms and Dry Ice)**

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 ship or transport class 6.2 and class 9 dangerous goods. Training validation is by successful completion of a quiz that includes evaluation and classification of substances to be shipped, labeling, packaging, and preparing shipping documentation. A shipping scenario exercise may be included as part of the quiz. A certificate of training is printed following successful completion of the training. This training is required annually. Training includes:

- IATA/ DOT Dangerous goods regulations;
- Classification of infectious substances; Genetically Modified Organisms and Microorganisms and Dry Ice
- Selection of proper packaging for shipping;
- Proper labeling and packaging of materials to be transported;
- Proper documentation needed;
- Safety and security during transport;
- Receiving packages.

**Facility Orientation Training/ Tour**

All persons should have this training.

**Lab Specific Training with PI/ Delegate**

This training is required and needs to be documented prior to working alone in the laboratory.

**Mock BSL3/Animal BSL3 (ABSL3)/ Arthropod Containment Level 3 (ACL3) Laboratory and Biosafety Cabinet Training**

All persons are required to have this training prior to working in a BSL3/ABSL3/ ACL3 laboratory. Persons are required to schedule this training with the Biosafety Office and LAR (if applicable). Training includes:

- Discussion of the biosafety levels, biosafety controls and biological risk groups.
- Biosafety practices and techniques;
- Facility entry;
• Barrier entry and exit;
• Putting on and taking off personal protective equipment (PPE);
• Use of respiratory protection;
• 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.
• Animal work specific procedures, if applicable.

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/ ACL3 laboratory**

This training is required by all persons prior to working in the BSL3/ABSL3/ ACL3 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, as applicable. This training requires an annual refresher.

**Barrier training inside the BSL3/ ABSL3/ ACL3 with a lab specific trainer**

This training is required and needs to be documented prior to working alone in the BSL3/ABSL3/ ACL3 laboratory. Basic BSL3/ABSL3/ ACL3 procedures, including those covered in the mock BSL3/ ABSL3 training, will be reviewed in the BSL3/ABSL3/ ACL3 laboratory setting. Training validation is by observation and review by the in barrier trainer and approval by the PI/ supervisor.

**Tier 1 and Select Agent and Toxin Regulations**

This training is required annually for all persons with select agent and/ or select toxin approval. This training must be taken prior to working in select agent/ toxin, BSL3/ABSL3/ ACL3 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 (including arthropods) as Select Agents;
• Loss, theft, or release of a Select Agent-Reporting Requirements;
• Inter- and intra-entity transfer of SAT;
• Inventory control;
• Insider threat;
• Tier 1 requirements.

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

SECTION 6- Occupational Health Program

All CSU employees working with biological agents are required to be enrolled in the CSU Occupational Health Program and complete the Risk Assessment Form annually by logging into:
http://www.ehs.colostate.edu/WOHSP/RiskAssessment/Add/AddRisAssessment.aspx

The Occupational Health Program includes medical surveillance and services 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.

More information related to the Occupational Health Program and additional resources can be found at: http://www.ehs.colostate.edu/WOHSP/Home.aspx

Respirator fit testing
Medical clearance, training and respirator fit testing (including N95 respirators) are required annually for individuals who are required to wear respirators for performing job tasks. Testing is performed using quantitative analysis.

SECTION 7 - Risk Assessment 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, arthropods, 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:
To prepare a risk assessment, individuals need to be familiar with:

- The organism:
  - Risks associated with working with each agent;
  - Infectivity;
  - Transmission;
  - Clinical symptoms;
  - Prevention (including vaccination) of disease;
  - Available treatments;
  - Infectious dose;
  - Stability in the environment;
  - Endemicity;
  - Susceptibility to disinfectants.

- The definition of the different Biological Safety Levels (BSL) 1, 2, 3 and 4, Animal Biological Safety Levels (ABSL) 1, 2, 3, and 4; Arthropod Containment Levels, Plant Biological Safety Levels…;

- 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 in the community. Risk Group assignment and Biosafety Level (containment) are not synonymous.

- Risk Group 1 microorganisms are not associated with disease in healthy humans or animals thus there is a low hazard risk.

- Risk Group 2 microorganisms are associated with human or animal disease for which preventive or therapeutic interventions are often available.
• Risk Group 3 microorganisms are associated with serious or life threatening human or animal disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).

• Risk Group 4 microorganisms are likely to cause serious or life threatening 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 found in the NIH Guidelines under Appendix B: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html#_Toc446948379

Publications of the occurrences of Laboratory Acquired Infections (LAIs) and historical accounts have provided the research community with important information that has 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 and 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 (BSCs)
- Airtight o-ring sealed rotors for centrifuges
- Mechanical pipettors
- Filter/ barrier tips
- Secondary transport containers
- Carts
- Autoclaves
- Puncture resistant sharps containers
- Plasticware
- Luer lock/ permanent needle and syringes
- Blunt end forceps/ scissors
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/ procedures
- Lab specific procedures
  - “Using a filter tip, pipet 10µl of X sample into 5ml of Y broth...”
  - “Check to make sure the orings are present in the bucket rotor(s) and balance the centrifuge...”

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
- NIH and BMBL “Guidelines”
- Select Agent Program Regulations

Proper Personal Protective Equipment:
Personal Protective Equipment (PPE) is the protective clothing worn for safety and may include:
- Close toed shoes
- Lab coat, back closing gown, coverall suit
- Respiratory protection
- Safety glasses, goggles or face shield
- Gloves
- Chemical Apron
- Appropriate attire and size

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 and Synthetic Nucleic Acid Molecules*. A copy of these publications can be obtained online at:


A biosafety level consists of a combination of laboratory design, required safety equipment, and practices and techniques, 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 level(s). 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 4 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:

a) The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

b) Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

c) 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.

d) Mouth pipetting is prohibited; mechanical pipetting devices must be used.

e) 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.

Precautions, including those listed below, must always be taken with sharp items.

1) 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.

2) Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

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

4) Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic-ware should be substituted for glassware whenever possible.

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

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

h) 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:

i) Must be placed in a durable, leak proof container and secured for transport.

ii) Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

i) 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.

j) An effective integrated pest management program is required.

2) 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.
- Work should be performed at Biosafety Level 1 (BSL1) or Biosafety Level 2 (BSL2). Infectious 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, using cell phones, etc. while in the laboratory; the use of mechanical pipettors; containment controls such as 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.

BSL-1:
1) Special Practices:
   a) Not applicable.

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 BSL2. BSL2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. *It differs from BSL1 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 BSL2:

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 organisms in a BSL2 laboratory.

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 need to 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, but are not limited to: 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 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.

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

Please Note: It is highly recommended that protective eyewear be worn while working in BSL2 labs, particularly when working with organisms that can be transmitted via mucosal routes and/or when conducting procedures that have the potential for creating splashes or sprays of infectious or other hazardous materials. Eye protection should be disposed of with other contaminated laboratory waste or decontaminated before reuse.

While it is ultimately the responsibility of the laboratory worker, there are certain situations when eye protection is required. When working in BSL2 laboratories, appropriate eye protection must be worn:

- While working with animals outside of the biosafety cabinet
- While transporting large volumes and/or high concentrations of broth cultures
- By persons who are wearing contact lenses

i) The type of eye protection worn is at the discretion of the laboratory worker and should be based on a risk assessment of the hazards; supervisors and other available resources should be consulted to select the appropriate protective eyewear.

e) Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse.

f) 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, BSL2 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.
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, depending on the specific class/type of BSC. 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 BSL3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL3:

1) Special Practices
   a) Same as BSL2.

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, scrubs, 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) Certain laboratory activities have the potential to generate aerosols, splashes, or sprays of chemicals or liquids containing infectious agents, toxins, or other hazardous materials, therefore posing a potential risk for exposure/injury to the eyes. The use of appropriate protective eyewear (i.e., safety glasses, goggles, face shield or other splatter guard) can help minimize the impact of this potential risk. It is the responsibility of the laboratory workers to understand the risks and use proper protective equipment when working in the lab.

Please Note: The Institutional Biosafety Committee highly recommends that protective eyewear be worn while working in BSL3 labs, particularly when working with organisms that can be transmitted via mucosal routes and/or when conducting procedures that have the potential for creating splashes or sprays of infectious or other hazardous materials. Eye protection should be disposed of with other contaminated laboratory waste or decontaminated before reuse. While it is ultimately the responsibility of the laboratory worker, there are certain situations when eye protection is required. When working in BSL3 laboratories, appropriate eye protection must be worn:

- While working with animals outside of the biosafety cabinet
- While transporting large volumes and/or high concentrations of broth cultures
- By persons who are wearing contact lenses

The type of eye protection worn is at the discretion of the laboratory worker and should be based on a risk assessment of the hazards; supervisors and other
available resources should be consulted to select the appropriate protective eyewear. Some examples of appropriate protective eyewear include (but are not limited to): safety glasses, goggles, face shields, and other splatter guards.

**Additional Resources**

CDC information on [https://www.cdc.gov/niosh/topics/eye/eye-infectious.html](https://www.cdc.gov/niosh/topics/eye/eye-infectious.html)

Product selection assistance and free samples: [www.northernsafety.com](http://www.northernsafety.com)

Colorado State University Occupational Health Program: EHS_Occhealth@colostate.edu

Colorado State University Biosafety Office: [https://vpr.colostate.edu/bso/](https://vpr.colostate.edu/bso/)

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. In addition, BSL3 laboratory workers should:

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

(2) Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

e) 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 BSL3 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 research involving animals:**

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

- **Arthropod Containment Guidelines**

- **Biosafety Considerations for Research with Lentiviral Vectors**

**SECTION 9 – Equipment and Laboratory Procedures**

**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 closed systems, 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, 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/ vents.

• All work should be performed at least ~4 inches from the inside edge of the front grille, in the middle, towards the back of the work space.

• 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.

• When needed, 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 an absorbent liner for infectious material work.

• Pathogen specific disinfectant and paper towels need to be placed on the clean side of the 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 the BSC.

• Serological pipets must be placed in durable containers, such as pipette boats 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 and allow the motor to get to the proper speed. 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 (UV) 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 and monitor the UV light in the BSC if one is in use.

References
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 or repaired.
- Annually.

Certification of a 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)

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. 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 that has been moved).

**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 ventilation racks 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 Office or Purchasing Agent in charge of the contract.

**CSU Sharps Policy**
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 or permanent needle and syringes.

Luer lock syringes are required to be used when needles with syringes are used or when needles are not permanently attached.

All sharps must be disposed of in the proper container(s) and must be labeled correctly 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.

The CSU Sharps Disposal Policy can be found under the “CSU EHS Occupational Health” tab or the Biosafety Office Website under the “CSU Sharps Procedures” tab:
- [http://www.ehs.colostate.edu/WOHSP/sharps.aspx](http://www.ehs.colostate.edu/WOHSP/sharps.aspx)

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

**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 shall be provided against pulling biohazardous aerosols or
overflow fluid into the vacuum system. This protection is provided by the use of a High Efficiency Particulate Air (HEPA) filter, an air filter in the line immediately leading into the house vacuum line and an overflow flask, containing pathogen specific disinfectant, for liquids between the collection flask and the air filter. The protection shall be provided by the laboratory using the vacuum system.

The overflow flasks need to 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.

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

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.

**Autoclave**

The autoclave uses steam, pressure and time to kill/ inactivate microorganisms by denaturing their proteins and/ or nucleic acids. Autoclaves are used to eliminate all microorganisms when appropriate conditions are met.

Safety Considerations need to be applied in order to safely use and validate an autoclave. These include (but are not limited to):

- Only trained personnel can operate the autoclave.
- Check the drain and make sure it’s clear.
- Never overfill the autoclave pans/bins or rack(s).
- Loosen plastic and glass container lids to prevent explosions in the autoclave.
- Place biological waste in autoclave approved bags labeled with the word “biohazard” and the biohazard symbol.
- Close the biohazard waste bag with autoclave tape and label the bag with autoclave tape containing:
  - Your first initial and last name
  - Date
  - PI’s last name
- Place materials in the autoclave and tape a sterility validation strip to the outside of the autoclave bag.
• Autoclave all biological waste materials for its appropriate sterilization time [Gravity displacement: 121°C (18-21 lbs. of pressure)]. Liquid Cycle (Slow exhaust, no vacuum dry).
  o The time should be adjusted based on the density of the load. Verification should be performed initially to confirm that the selected sterilization time is sufficient.
• Use a log to record autoclave validation.
• Autoclaved material must be allowed to cool sufficiently before removal from the autoclave or opening containers, to reduce the possibility of steam burns or boil over of liquids.
• If an alarm is activated, abort the run, note the error code and contact your building proctor/ Operations group for maintenance.
  o Make note of the alarm in the autoclave log book.
  o Post a note on the autoclave indicating it is down and not to use it.
• Stand off to the side when opening autoclave door.
• Wear proper PPE (autoclave gloves, apron, eye protection) when loading/unloading the autoclave.
• Materials that cannot be autoclaved
  o Certain chemicals- check with EHS Chemical Management if unsure.
  o Radioactive materials
  o Equipment intended for reuse or servicing (i.e. freezers, computers, centrifuges, etc.)
• Do not overload the autoclave.
• Make sure the disinfectant/ chemicals used with the biological material are safe to go into the autoclave.

Biohazard Autoclave Bags in BSC
• Pour liquid (pathogen specific disinfectant/water) into each bag before the bag is used.
• When bag is 2/3 full of waste, seal the bag using autoclave tape.
  o Seal the bag tightly to prevent bag from pushing air from inside to the outside.
  o Visually inspect the bag for any holes or sharp items that may be able to penetrate through the bag.
• Wipe down autoclave bag with disinfectant and remove it from the Biological Safety Cabinet (BSC).
• On a piece of autoclave tape label legibly with a thick black permanent marker:
  o PI’s last name
  o Your first Initial and complete last name
  o Date
• Place the piece of autoclave tape onto the sealed autoclavable biohazard bag.
• Place the autoclavable biohazard bag in an autoclave bin and;
  o Place the labeled waste in the specific autoclave staging area or
• Place the autoclave bin with the labeled waste directly into the autoclave.
• Additionally:
  o Animal carcasses and agar plates need to be double bagged.
    ▪ Ziploc style bags count as one bag.

**Animal carcasses that need to be autoclaved**

  o Animal carcasses and other animal material need to be bagged in biohazard bags and completely thawed (at least 48 hours) before they are placed in the autoclave.
    ▪ It is the laboratorian’s responsibility to ensure that the animal carcass(es)/animal material is completely thawed.
  o Animal carcasses/animal material needs to lie flat and be a single layer in the biohazard bag.
  o Whole eggs are frozen to euthanize the embryo. It is the responsibility of the researcher to euthanize the embryo, then place the egg(s) in the refrigerator for at least 48hrs before staging the eggs for autoclaving.

**Loading the autoclave (see building specific Autoclaving SOPs)**

• Do not stack one piece on top of another.
  o The only exception is pipette boats which can be stacked two high.
• Place the autoclave bags in autoclave pans/bins to contain leaks.
• Waste should be bagged and labeled appropriately (see above).
• Bag opening should be facing upward, when possible.
• Laundry can go in without a pan/bin.
  o One to two Sterigages are to be placed with the load.
  o Placement may vary according to building specific Autoclave SOP
• Label Sterigage as appropriate:
  o Date
  o Time
  o Cycle
  o Location
  o Your initials
• Log results/ the chemical indicator in an autoclave verification log.
  o Check the drain to make sure it is clear; clean it when it’s dirty.
  o Push the cart into the autoclave, if applicable.
  o Close/lock the autoclave door.
    ▪ Make sure that you move the handle from open back to close/locked (on units that have locking door handles).
  o Start the autoclave.

Standard cycles may be programmed into the autoclave (see building specific Autoclaving SOPs)
Biological Indicator Verification (see building specific Autoclaving SOPs):
• At least once every 6 months (or as appropriate per building / autoclave specific SOPs) a biological indicator/spore test is to be placed in the autoclave load.
  o The best location to place the biological indicator/ spore test is on the bottom shelf, near the door, and over the drain of each autoclave.
  o Label the biological indicator/ spore test with the date, time, cycle, autoclave location and your initials, as appropriate.
  o When the autoclave cycle is complete, the biological indicator/ spore test is removed and the biological indicator/spore test instructions are followed.
    ▪ For use of the Verify® Spore test (SCBI), when the autoclave cycle is complete, the spore test is removed and incubated at 55-59⁰C within *two hours of the end of the cycle along with a control spore test from the same box for at least 24 hrs.
      • If the Verify® Spore test is not going to be incubated immediately, it is recommended that it not be activated, but kept in a sealed condition at room temperature for up to 48 hours following completion of the sterilization cycle. After the 48 hour window, the Verify® Spore test will be activated and incubated at 55-59⁰C for at least 24hrs along with a control spore test from the same box.
  o Log the biological indicator/ spore test results in an autoclave verification log.

Unloading the autoclave (see building specific Autoclaving SOPs):
• Verify that the cycle was properly completed from the printed output and the display screen.
  o Record any discrepancies or problems in the autoclave logbook or on the autoclave alarm log, if applicable.
• Verify the chamber pressure reads 0 PSI.
• Stand to the side of the autoclave door and open.
• Verify the chemical indicator (Sterigage) test strip has successfully passed.
  o If not, the cycle will need to run a second time.
  o Contact your manager/Operations if this happens.
• When using a trolley to load the autoclave, line up the trolley to the autoclave and make sure it latches firmly.
• While wearing proper PPE, pull the cart out of the autoclave until it firmly latches onto the trolley.
• Remove the Sterigage from the load and place into the autoclave verification logbook.
• Fill in the appropriate areas of the autoclave logbook.
• When using a cart, slide it back into the autoclave.
• Disengage the trolley, when applicable.
• Close/lock the autoclave door if it is an interlock system.
  o Make sure that you move the handle from open back to close/locked
  (on units that have locking door handles).

Contingencies
• All users must leave the autoclave accessible at all times.
• If you run a load, you are responsible for unloading the materials before the next
  user’s autoclave schedule starts.
  o Only exception is if the autoclave starter has communicated with
  someone else in their party to have the autoclave unloaded when the
  cycle is complete.

Centrifuge and Containment
The laboratory may be equipped with microcentrifuges, low-speed centrifuges,
conventional centrifuges and/or ultracentrifuges (with various tube-rotor combinations).
Safety precautions must be followed when using these centrifuges including:
• Air-tight sealing rotors, aerosol canisters / safety cups, or other engineering
  controls, when and where required for safety from aerosol production.
  o Check and make sure o-rings, lids, and tubing are not cracked or missing.
• Each operator must be trained on the proper operating procedures.
  o Follow manufacturer’s instructions on usage.
• A log sheet detailing safety and maintenance checks for centrifuges and rotors
  must be maintained.
• Tube selection.
  o Assure that the microcentrifuge or centrifuge tube is made of the proper
    material for the application and chemicals it will be holding.
  o Make sure they are rated for the speeds at which you will be using them.
• Place the rotor or bucket in the centrifuge.
• Do not exceed safe rotor speed.
• Place a biohazard label on the centrifuge if used for infectious agents.
• Always use the appropriate sealed safety buckets or sealed rotors with O-rings,
  as applicable to the work being performed in the centrifuge.
• Remember that swinging bucket rotors have well-defined bucket placement
  holes that are numbered. The hole number must match the bucket number. Do
  not place buckets in a wrong rotor hole! Do not run the rotor with fewer than
  three buckets. If you have only two filled buckets, use also two more empty
  buckets, placing them opposite each other in their appropriate rotor holes.
• Match the serial number of each swinging bucket to the identical serial number
  of the corresponding rotor.
• Load and unload safety buckets or rotors within the biosafety cabinet, as
  applicable.
• Check tubes and bottles for cracks and deformities before each use.
• Examine O-ring and replace if worn, cracking or missing.
• Never under fill or overfill primary containers; do not exceed ¾ full.
• Wipe exterior of tubes or bottles with disinfectant prior to loading into safety buckets or rotor.
• Balance opposing tubes carefully - including the lid.
• Decontaminate safety buckets or rotors and centrifuge interior after each use.
• Watch the centrifuge until it has reached the correct speed. Stop the centrifuge immediately if an unusual condition, such as noise or vibration, begins.
• If secondary containment (ie. Safety bucket rotors or an airtight rotor) is not available, wait five minutes after the run before opening the centrifuge in the BSC to allow aerosols to settle in the event of a breakdown in containment.
• After centrifuge run is complete, take out the rotor or bucket(s), if you are able to, and place it in the BSC.
• Open the rotor or bucket lid in the BSC and clean out the rotor or bucket using appropriate disinfectant.
• Wipe the exterior of safety buckets or rotors with disinfectant before removing from biosafety cabinet.
• Care should be taken to avoid contaminating the cap or tube screw-threads with liquid.

Storage: Shelves/ Incubators/ Refrigerators/ Freezers

Safety Considerations:
• To reduce spillage and reduce confusion, materials should be stored in an orderly fashion, labeled correctly, and transferred to and from storage locations in secondary containers.
• An inventory log may be useful. This log could have fields for initial Date, Name, Pathogen(s), and User initials, removal and/or destroyed date on the storage door or container.
• All biological materials should be clearly labeled with: a. Genus, b. Species and any other identifier ie. ATCC or TMC #, c. Date, d. owners initials. Other pertinent information can be included.
• Inoculated plates should be placed in secondary container(s) and transported to the storage location.
• Plastic boxes or secondary containers can be labeled on the outside of them if they are being used for storage purposes.
• Material on shelves should be stored away from the edge.
• Check cultures regularly to avoid unnecessary clutter.
• Dispose of contaminated material safely and appropriately as you don’t know what the contamination may be.
Water baths
- A lid, if provided, should always be on the water bath.
- The water bath should be filled with distilled water to about the 1/3 full level.
- Water should be changed periodically to avoid growth of bacteria etc.
- If you must change the temperature of a water bath, put a note on the lid with your name and change the water bath back to its previous temperature after use.
- Put a note on the lid with name and anticipated time of use if you are going to be leaving the water bath for an extended amount of time.
- Make sure the thermometer range is suitable i.e. do not leave the water bath at a high temp.
- Use a stand-alone thermometer to verify waterbath temperature.
  a. Do NOT use mercury thermometers.

Lab Specific Equipment
See your lab specific equipment manuals and SOPs.

SECTION 10- Biological Decontamination

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 working concentrations of disinfectant in the laboratory. Follow the manufacturer’s instruction for dilution and use, unless approved experiments have been performed on the specified organism. Supervisors must devise schedules for disposal of ineffective residual decontaminants and replenishment with fresh solutions. For spill cleanup, personal supervision of the application to the spill area of a known effective chemical disinfectant in sufficient concentration with adequate contact time may be a criterion selected for allowing research to be resumed following a spill.

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 Management) must annually perform periodic testing and maintenance of these units.

Users of autoclaves and steam sterilizers shall be trained on how to use the autoclave correctly and shall use chemical/temperature indicators with each load of material to be sterilized. Documentation of the use and verification of the indicators that the autoclave functioned correctly shall be maintained by the autoclave users and provided upon request. Biological indicator validation tests should be performed at least every 6 months for all autoclaves and documentation maintained. Autoclaves in BSL3 areas need to use biological indicators more frequently as directed by Building Directors and the Biosafety Office. 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 Management or a contractor) must be trained in maintenance and testing to the satisfaction of the BSOs and/or the 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 any necessary repairs are completed and the unit is recertified.

**Vaporized Hydrogen Peroxide (VHP)/ Gaseous Chlorine Dioxide Decontamination**

VHP and Chlorine Dioxide decontamination is conducted by trained and authorized personnel when biologically hazardous equipment is too large or sensitive for autoclaving. VHP and Gaseous Chlorine Dioxide can only be used on materials that have smooth, clean, non-absorbent surfaces. Oxidizers can be tough on many materials. Both chemical and biological indicators shall be used to test and validate the decontamination process.

Compatibility of Material and Electronic Equipment with Hydrogen Peroxide and Chlorine Dioxide Fumigation:

file:///C:/Users/hab26/Downloads/COMPATIBILITY%20OF%20MATERIAL%20AND%20ELECTRONIC%20EQUIPMENT%20WITH%20HYDROGEN.PDF

**Paraformaldehyde**
Paraformaldehyde may be used to fumigate Biological Safety Cabinets or areas that are judged not to be reliably decontaminated using surface wiping or another decontaminating method. Gas is the most penetrating type of delivery mechanism for a sterilizing chemical. Because of this property, it is typically used in applications when debris in the area cannot be thoroughly cleaned up, or there are areas where penetration is required into hard to reach areas.

**Pros**
- Widely accepted due to long time use and familiarity
- Efficiently inactivates a broad spectrum of organisms
- Economical
- Powerful decontaminant
- Non-corrosive to metals
- Relatively easy to generate

**Cons**
- Probable human carcinogen
- Neutralization forms white residue which must be cleaned after decontamination
- Acute respiratory irritant
- Must neutralize
- Requires high relative humidity

**Tissue Digestion**
Biological digesters may use agitation and heat to break down tissue with alkali to amino acid chains or may use a combination of water, caustic, and heat to create the environment for hydrolysis to decompose animal carcasses and other biological hazardous waste materials safely and rapidly. The bio-digestion is less harmful to the environment than other methods.

**Effluent Decontamination System/ Kill tank**
Effluent Decontamination Systems (EDS) may be used to decontaminate biohazardous liquid waste containing solids that may go down the sink, shower and/or floor drain(s). This system uses heat to “cook” the biohazardous liquid and solid waste to ensure that the material is decontaminated before disposal to the sewer.

**Incineration**
Incineration is a waste treatment option that involves the combustion of organic waste material using high temperature that converts the waste into ash, flue gas and heat.
Section 11- 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 the infectious organism(s) in use in the laboratory.

Incident Investigation
Incidents in laboratories and/or clinics and infections resulting from such work with infectious agents must be promptly reported to the Biosafety Office. 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/ Accidents
All biological incidents/ accidents must be reported immediately to the laboratory PI/supervisor and to the Biosafety Office. Such incidents include but are not limited to personnel exposures, injuries, release of biohazard material(s) and failure of biological containment. The PI/supervisor must assist Biosafety 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 Biosafety Office, 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 recombinant or synthetic molecules.

The investigation of all incidents associated with infectious agents or recombinant research will include a review of techniques and procedures as well as types and uses of equipment that may have been involved in the accident/ incident. The investigation will identify/ determine the circumstances leading to the accident/ incident.

The Policy on Biosafety and Biohazards non-compliance has been posted on the ORCC/ IBC website: https://www.research.colostate.edu/orcc/ibc/policies-and-regulations/

In addition, the investigation report, by the Biosafety Office/ PI/ Institutional Biosafety Committee (IBC), should provide recommendations for preventing similar occurrences.

All incidents or accidents shall be reported as follows:
- Each person involved in biological work shall report to his/her PI/supervisor and
to the Biosafety Office:
  - 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, needs to report it to the Biosafety Office within 24 hours.

• When an employee is injured while performing job duties, medical evaluation may be sought. 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.

  o Students or volunteers that are not paid for their biological work should seek medical evaluation through CSU Health Network or personal care physician in the event of injury.

• Each person/supervisor/department is responsible for reporting all claims to EHS/Risk Management and Insurance within four (4) working days (http://rmi.prep.colostate.edu/insurance/incident-reporting/). Special reports may be required to properly document the incident for compensation, statistical and accident prevention purposes. Risk Management may be contacted for clarification and assistance in this requirement (970-491-6745).

• 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:
  o Fatality.
  o Hospitalization or medical treatment (beyond first-aid) NOTE: This includes non-CSU personnel.
  o Property damage exceeding $1,000.00.
  o Biological exposure resulting in accidental release of biohazards outside of containment. Also contact the Biosafety Office (491-0270).
  o Infectious Material Incidents (including recombinant and synthetic nucleic acid molecules and infected animals). Also contact the Biosafety Office (491-0270).

• All incidents involving infectious materials are to be immediately reported to the Biosafety Office. Such incidents may include spills or releases of materials or agents, recombinant and synthetic nucleic acid molecules, escape of infected
animals, rupture of plastic bags of infectious/medical waste, or other loss of containment. The Biosafety Office and/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). Inform them:
  - Where and what type of incident has occurred.
  - Nature and type of any injured or trapped persons.
  - What has happened since the incident occurred: 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 Biosafety Office, 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/ Biosafety 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, or perform temporary fixes to 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, and freezers 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

Complete emergency contact information can be found at:
www.ehs.colostate.edu/WEmgResp/Home.aspx
Complete list of Current Medical Providers: http://rmi.prep.colostate.edu/workers-compensation/authorized-treating-physicians/

FIRE or LIFE THREATENING EMERGENCY – 911

UNPAID STUDENTS AND VOLUNTEERS
Seek medical attention from CSU Health Network or your personal health care provider.

CSU Biosafety and Occupational Health are available to facilitate consult

BIOSAFETY OFFICE
Biosafety Emergency: 491-0270

ENVIRONMENTAL HEALTH SERVICES
491-6745

Occupational Health: 420-8172
Radiation Control: 491-4835
Chemical/ Haz. Waste: 491-4830

RISK MANAGEMENT/WORKERS’ COMPENSATION
491-6745

Online injury report: http://rmi.prep.colostate.edu/workers-compensation/file-an-incident/

Injury Report Instructions: http://rmi.prep.colostate.edu/workers-compensation/incident-procedure/

Fort Collins Medical Providers
When possible, employees should seek medical attention with one of the following authorized physicians:

UC Health Occupational Health (Mon-Fri 8AM-5PM):
• CSU Campus
  o 151 W. Lake St, first floor
  o 970-237-8250
• South Location
  o 2315 Harmony Rd, Ste 170
  o (Redstone Building)
  o 970-495-8250

Workwell (Mon-Fri 8AM-5PM)
  o 1600 Specht Point Road Suite 115
  o (970) 672-5100

After Hours Urgent Care
Mon-Fri 5PM-8PM; Sat-Sun 9AM-5PM
Associates in Family Medicine CSU
  o 151 W. Lake St, first floor
  o 970-237-8200

After Hours and Severe Injuries
24 hours a day, 7 days a week
Poudre Valley Hospital Emergency Department
1024 South Lemay Avenue
(970) 495-7000

UC Health Emergency Room (24/7)
4630 Snow Mesa Drive
(970) 237-8100

Priority One - Always Seek Medical Attention
SECTION 12– Spill Clean Up

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

BSL1 and BSL2 Laboratory Spill Cleanup

Recommended Biological Spill Kit Contents
- Gloves; latex and nitrile or appropriate for the spill
- Lab coat; disposable gown
- Respirator or Face shield
- Eye protection
- Booties to protect shoes
- Towels/ absorbent material
- Pathogen specific disinfectant
- Brush, dustpan; tongs
- Puncture-proof container for sharps
- Tape
- Marker pen
- Biohazard labeled 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 the Biosafety Office or 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 pairs of 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 30 minutes or pathogen specific disinfectant contact time)
• Starting from outside of spill area, clean up towels moving inward
• Dispose of towels in biohazard bag
• Seal the biohazard bag with tape
• Repeat process
• Mop surrounding area (10 feet on each side), Autoclave and/or contact the Biosafety Office or EHS for disposal (491-0270; 491-6745)
• If You Are Exposed
  o Clean all exposed skin with soap and water for 3-5 minutes
  o Rinse mucous membranes or eyes with water for 15 minutes
  o Record the location and time of incident
  o Report the incident to your PI/supervisor and to the Biosafety Office
  o Seek evaluation at University's medical provider
  o Fill out an incident report (https://drive.google.com/file/d/0BzRT2XYL3jhNVJxT3JxSUdrOUk/view) within 24 hours and Worker’s Compensation form (http://rmi.prep.colostate.edu/workers-compensation/file-an-incident/) 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 pathogens. For some of these, ten or fewer viable particles of certain pathogens 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 (chemical and biological) or research (biological) Powered Air Purifying Respirator (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.
- Shout to notify all personnel within the room that a spill occurred and everyone should exit lab area.
- Post ‘DO NOT ENTER – Spill Cleanup In Progress’ sign located in the spill kit (figure 1.0).
- Now Shower Out placing clothing in the autoclave trash bin.
- Post ‘Spill Cleanup’ (figure 2.0) signs located in locker room on the outside of the men’s and women’s locker room doors.
- Call 491-0270 (Biosafety).
  - Always Remember ESPNPC.
  - Wait to be accompanied by Biosafety.
- Wait 1 hour before returning to the spill site.
  - You should be accompanied by a Biosafety Officer.
  - Put on regular PPE and Spill Cleanup PPE:
    - Tyvek coverall
    - Two pairs of gloves (outer gloves over Tyvek sleeves)
• Emergency PAPR for hazardous chemical and biological spills. Research PAPR for biological spill, no hazardous chemicals.

• Fill mop bucket up with pathogen specific 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 according to the Autoclavable Biohazardous Waste Disposal SOP.
• Mop a 10 ft. radius from the clean side to dirty side of the room, using pathogen specific disinfectant.
  • Wipe down cabinets, benches, walls, etc. with disinfectant.
• Remove mop head, place into autoclave bag and wipe down mop handle with pathogen specific 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 and label 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 their original location.
• Remove ‘Spill Cleanup -In Progress’ sign.
• Shower out.
• Remove locker room signs.
• File a Biosafety Incident Report Form within 24 hours. Expect to review the incident with your PI/Supervisor 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 after consultation with EHS'-Hazardous Waste group (e.g. acid spill kit).

• If radioactive material 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 (fabric like paper towels - hold together better)

Procedure
If the spill is contained on the absorbent liner:
• Carefully and slowly remove materials away from the spill to the “dirty” side.
  o 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.
• Slowly fold up the spill in the liner and place gently into the autoclave bag inside the BSC.
• Remove gloves and put on new pair or if double gloved, remove outer layer of gloves and put on a new layer.
• Replace materials and resume working in BSC.

If the spill is NOT contained on the absorbent liner:
• Cover the spill with paper towel(s).
• Replace gloves or replace outer layer of gloves with a new pair if double gloved.
• 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.
• 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 disinfectant technical report).
• Place materials that were splattered by the spill in an autoclave bag or in a liquid catching container.
  o Replace (outer) gloves accordingly
• 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.
  o Replace (outer) gloves accordingly
• Thoroughly wipe down the internal surfaces of the cabinet with pathogen specific disinfectant.
• 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/vent area (KEEP THE CABINET RUNNING):
• Unscrew the lid of pathogen specific disinfectant and flood the BSC front grille/vent (or back grille/vent if the spill began there) area with the disinfectant.
• Clean up spill on top (work area) according to Procedure 2 (above).
• Notify Biosafety (491-0270)
• Contact an individual (a lab mate or biosafety officer) to help with the cleanup process.
• Wipe down the countertop where you will be placing the cleaned BSC parts.
• Using 3 or four pieces of autoclave tape, place a new autoclave bag on the inner side of the BSC wall for the Biohazardous waste.
  o This will allow you to keep the bag in the BSC, but will not be in the way when it is time to remove the stainless steel work area (See Figure 1).
  o Do not block the front or back grills/ vents.

As a team:
• 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.
• 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.
• Using the Swiffer or equivalent tool, wipe up the underside of the work area part of the BSC/ the pool of disinfectant under the work area.
  o DO NOT USE YOUR HANDS as sharps ARE present!
  o Have the person not in the BSC assist with providing new paper towels.
  o Replace (outer) gloves accordingly
• Wipe down the autoclave bag with pathogen specific disinfectant, seal the autoclave bag and remove the bag from the BSC.
• Wipe down the BSC parts again with disinfectant and place the BSC parts back into the BSC accordingly.

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 needed
whenever transporting liquid material and is mandatory for transit of infectious materials within the corridors serving the laboratories. Individuals that are transporting potentially infectious materials (diagnostic specimens, cultures, etc.) between different sites (VTH, Foothills campus, etc.) at CSU or transferring these materials to other entities or institutions must complete the Shipping Training unit and comply with the requirements for packaging, labeling, and transporting all packages. Trained persons transporting infectious materials to other CSU sites must carry a Bill of Lading signed by a BSO, their laboratory’s Spill Response Plan, and their certificate of Shipping Training during transport.

International Air Transport Association (IATA) and Department of Transportation (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, exempt human and animal specimens (diagnostic samples),
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**

**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 the 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 an infectious substance, the Biosafety Officer (Responsible Official/ Alternate Responsible Official) immediately contacts the CDC and a report is filed with them.

**Shipping and Receiving Select Agent/ Select Toxin**
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. All provisions of these final rules superseded those contained in the interim final rules.

IATA/DOT Dangerous Goods 6.2 training certification and Security Risk Assessment (SRA)/ Tier 1 (if applicable) approval is required for shipping or receiving Select Agents/ Tier 1 select Agents internally or externally. IATA/ DOT Dangerous Goods 6.1 training
certification and Security Risk Assessment (SRA)/ Tier 1 (if applicable) approval is required for shipping or receiving Select Toxins.

The PI and Responsible Official/ Alternate Responsible Official (one of the Biosafety Officers) are responsible for completing the required documentation before preparing the package for shipment. See Facility specific Biosafety, Security and Emergency/ Incident Response Plans regarding shipping and receiving select agent/ toxin.

**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:**
• Smallpox virus
• 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, Equine infectious anemia 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).