Introduction

The Institutional Biosafety Committee (IBC) requires that the following standard and special microbiological practices, physical containment or laboratory design, containment equipment, and training be implemented when research or teaching activities involve the use of biohazards, recombinant DNA molecules (rDNA), select agents, or bloodborne pathogens. These requirements include hygienic and operational practices that are critical in providing for a safe work environment and assuring a viable research product is produced. These practices are also necessary for minimizing and/or eliminating the risk of occupational exposure to infectious and potentially infectious substances.

The Principal Investigator is responsible for having their laboratory area meet the specified requirements for the Biosafety containment Level that corresponds to the biological agents in use. Failure to meet these requirements will result in a review by the IBC. The Principal Investigator will work with the IBC to correct all deficiencies in a timely manner.

Currently, the CU Boulder Campus (UCB) conducts research that requires Biosafety Level 1 (BL1) and Biosafety Level 2 (BL2) physical containment only. Therefore, only the descriptions and assignments of physical containment for BL1 and BL2 are detailed below.

The NIH Office of Biotechnology Activities (NIH-OBA), which is responsible for the NIH system of oversight of recombinant DNA research, human gene transfer research, and Duel-Use research developed a set of NIH guidelines that would govern the safe conduct of recombinant DNA research by outlining appropriate biosafety practices and containment measures. These guidelines, known as the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), can be found at: [http://oba.od.nih.gov/rdna/nih_guidelines_oba.html](http://oba.od.nih.gov/rdna/nih_guidelines_oba.html)

While compliance with the NIH Guidelines is mandatory for investigators at institutions receiving NIH funds for research involving recombinant DNA, they have become a universal standard for safe scientific practice in this area of research and are followed voluntarily by many companies and other institutions not otherwise subject to their requirements.

In addition to the NIH Guidelines, the IBC references the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) as a standard of practice for biosafety when addressing the safe handling and containment of infectious microorganisms and hazardous biological materials.

The CDC/NIH Biosafety in Microbiological and Medical Laboratories, 5th Edition can be found at: [http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm)
Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices. Consequently, all personnel directly or indirectly involved in experiments using rDNA shall receive adequate instruction. At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided.

Physical Containment Levels

The objective of physical containment is to confine organisms containing rDNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing rDNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment that are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. There are four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. The National Cancer Institute describes three levels for research on oncogenic viruses that roughly correspond to NIH BL2, BL3, and BL4 levels. Currently, UCB conducts research that requires BL1 and BL2 physical containment only. Therefore, only the descriptions and assignments of physical containment for BL1 and BL2 are detailed below.

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given to other combinations that achieve an equivalent level of containment.

Biosafety Level 1 (BL1)

BL1 Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
- Work surfaces are decontaminated at least once a day and after work with infectious
materials is finished, and after any spill of viable material is cleaned with disinfectants that are effective against the agents of concern.

- All contaminated liquid or solid wastes are decontaminated before disposal in accordance with the UCCS “Biological Laboratory Waste Management-Disposal Policy and Procedure”.

- Mechanical pipetting devices are used; mouth pipetting is prohibited.

- Policies for the safe handling of sharps are instituted. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, and in accordance with the UCCS “Biological Laboratory Waste Management-Disposal Policy and Procedure”.

- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. According to Environmental Health and Safety (EH&S) Laboratory Guidelines, no preparation, storage or consumption of food or drink is permitted in the lab.

- Persons wash their hands:
  - after handling materials involving organisms containing rDNA molecules and animals
  - before exiting the laboratory

- All procedures are performed carefully to minimize the creation of splashes or aerosols.

- In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, and changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing rDNA molecules.

- A biohazard sign must be posted at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and the phone number of the investigator. Please see last page of this document for an example of an appropriate biohazard notification sign.

**BL1 Special Practices**

- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container that is closed before being removed from the laboratory.

- An insect and rodent control program is in effect.

**BL1 Containment Equipment**

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

- Gloves should be worn if the skin on the hands is broken or if a rash is present.

- Protective eyewear should be worn for conduct of procedures in which splashes of
microorganisms or other hazardous materials is anticipated.

**BL1 Laboratory Facilities**

- Laboratories should have doors for access control.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing. Foot, knee, or automatically operated sinks are recommended.
- If the laboratory has windows that open, they are fitted with fly screens.

**Biosafety Level 2 (BL2)**

**BL2 Standard Microbiological Practices**

- All procedures for BL1 Standard Microbiological Practices, AND
- Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

**BL2 Special Practices**

- All BL1 Special Practices, AND
  - The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections.
  - The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.
  - When the organisms containing rDNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent and the biosafety level, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering and exiting the laboratory (e.g., immunization, personal protective equipment). Please see last page of this document for an example of an appropriate hazard warning sign.
Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Animals not involved in the work being performed are not permitted in the laboratory.

Special care is taken to avoid skin contamination with organisms containing rDNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent, contaminated surfaces or equipment is unavoidable. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Hands are washed following removal of gloves.

All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain rDNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, and in accordance with the UCCS “Biological Laboratory Waste Management-Disposal Policy and Procedure”.

Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Broken glassware should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, and in accordance with the UCCS “Biological Laboratory Waste Management-Disposal Policy and Procedure”.

Spills and accidents that result in overt exposures to organisms containing rDNA molecules are immediately reported to the IBC. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Laboratory personnel receive appropriate immunizations or tests for the agents handles or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

The Principle Investigator ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary
precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

BL2 Containment Equipment

- **All BL1 Containment Equipment, AND**
- Properly maintained biological safety cabinets (Class I or II), preferably Class II, or other appropriate personal protective or physical containment devices are used whenever:
  
  1. Procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.
  
  2. High concentrations or large volumes of organisms containing rDNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

- A properly maintained biological safety cabinet (Class I or II), will have a current, annual certification that under normal operating circumstances the unit performs to Manufacturer’s specification. A list of qualified Vendors who can perform the required calibration and certification can be obtained from Environmental Health & Safety. Provide a copy of BSC certification report to EH&S, Public Safety.

- Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the biological safety cabinet.

BL2 Laboratory Facilities

- **All BL1 Laboratory Facility Requirements, AND**
- Provide lockable doors for facilities that house restricted agents.

- Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets’ air flow parameters for containment.

- An eyewash station is readily available.

- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

- An autoclave for decontaminating laboratory wastes is available.
Biosafety - EMS.IBC Lab Requirements for BL1 and BL2 Containment

Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. For additional information on biological safety cabinets, please visit: CDC-NIH Selection, Installation and Use of Biological Safety Cabinets.

**Class I** - The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g. cage dumping, culture aeration or tissue homogenation).

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the cabinet exhaust plenum. A second HEPA filter may be installed in the terminal end of the building exhaust prior to the exhaust fan.

**Class II** - Class II BSCs are partial barrier systems that rely on the laminar movement of air to provide containment. If the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms) the potential for contaminant release into the laboratory work environment is increased as is the risk of product contamination.

The Class II (Types A1, A2, B1 and B2) BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection. Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a hard connection.

HEPA filters are effective at trapping particulates and thus infectious agents but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1 and B2 BSCs exhausting to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan.

**Class III** - The Class III BSC was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained
by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure. The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility ventilation system.

**Horizontal Laminar Flow “Clean Bench”**

Horizontal laminar flow “clean benches” are not BSCs. These pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled. Horizontal airflow “clean benches” must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these two devices.

**Vertical Laminar Flow “Clean Bench”**

Vertical laminar flow clean benches also are not BSCs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems presented by the horizontal laminar flow clean benches. These benches should never be used for the manipulation of potentially infectious or toxic materials.

**Comparison of Biosafety Cabinet Characteristics**

<table>
<thead>
<tr>
<th>BSC Class</th>
<th>Face Velocity</th>
<th>Airflow Pattern</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonvolatile Toxic Chemicals and Radionuclides</td>
</tr>
<tr>
<td>I</td>
<td>75</td>
<td>In at front through HEPA to the outside or into the room through HEPA</td>
<td>Yes</td>
</tr>
<tr>
<td>II, A1</td>
<td>75</td>
<td>70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit</td>
<td>Yes, minute amounts</td>
</tr>
<tr>
<td>II, B1</td>
<td>100</td>
<td>30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter</td>
<td>Yes</td>
</tr>
<tr>
<td>II, B2</td>
<td>100</td>
<td>No recirculation; total exhaust to the outside through a HEPA filter</td>
<td>Yes</td>
</tr>
<tr>
<td>II, A2</td>
<td>100</td>
<td>Similar to II, A1, but has 100 fpm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to the outside through a canopy unit</td>
<td>Yes</td>
</tr>
<tr>
<td>III</td>
<td>N/A</td>
<td>Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

2. In no instance should the chemical concentration approach the lower explosion limits of the compounds.
BIOHAZARD NOTIFICATION

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Custodial Staff Can:

☐ Empty Trash
☐ Clean Floors

Entry By:
☐ Lab Staff
☐ Facility Management
☐ Emergency Personnel

Risk Group
☐ 1
☐ 2

Biosafety Level
☐ 1
☐ 2

Biological Agents:

Risk Group
☐ 1
☐ 2

Biosafety Level
☐ 1
☐ 2

Biological Agents:

Risk Group
☐ 1
☐ 2

Biosafety Level
☐ 1
☐ 2

Biological Agents:

Risk Group
☐ 1
☐ 2

Biosafety Level
☐ 1
☐ 2

Special Lab Entry Requirements:

Special Lab Exit Requirements:

In Case of Emergency Contact:

Principal Investigator

Work Phone # ____________________________
Home Phone # ____________________________

Lab Contact

Work Phone # ____________________________
Home Phone # ____________________________