Contents

1 Purpose

This manual provides biosafety guidelines for those working at The University of Colorado Colorado Springs (UCCS) including any work that involves the handling of:

1. biohazardous materials
2. recombinant or synthetic nucleic acid molecules (rsNA)
3. human or animal fluids, tissues, or cell lines

This biosafety manual has been developed by the Environmental Health and Safety (EHS) at UCCS. The manual is part of UCCS's biosafety program established to accomplish the following goals:

1. protect personnel from exposure to infectious agents
2. prevent environmental contamination
3. provide an environment for high quality research while maintaining a safe workplace
4. comply with applicable federal, state, and local requirements

The biosafety plan provides university-wide safety guidelines, policies, and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI), its success depends largely on the combined efforts of the laboratory supervisors and employees. Planning for and implementation of biological safety must be a part of every laboratory activity in which biohazardous materials are used.

In general, the handling and manipulation of biological agents and toxins, as well as recombinant or synthetic nucleic acid molecules, requires the use of various precautionary measures depending on the material(s) involved. This manual will provide assistance in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary.

2 Instructions

EH&S will be responsible for updating the manual periodically to reflect changes in relevant guidelines, regulations, and policies as they occur. Researchers will be notified when significant changes have been made.

Suggestions for researcher generated documents that should be added to this manual to enhance its usefulness are:

1. Current IBC Biosafety Application
2. Standard Operating Procedures for:
    a. Decontaminating laboratory surfaces
    b. Addressing spills of biological materials
    c. Biosafety cabinet operation
    d. Autoclave operation

e. Specialized equipment operation and maintenance unique to the research

3 Principals of Biosafety/Biosafety Levels

3.1 General Elements of Containment

Biosafety in Microbiological and Biomedical Laboratories (BMBL) https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF , published by the United States Department of Health and Human Services, is the definitive reference on biosafety and should be read and followed by all UCCS personnel working with potentially infectious agents.

Central to any discussion involving biosafety is the concept of containment of infectious agents to prevent contamination of the worker, nearby workers, or the environment. Containment is also utilized to prevent contamination of research samples or animals. There are three general elements of containment:

1) Laboratory practices and techniques
2) Safety equipment
3) Facility design

Each of these will be discussed briefly – for more detail, see the section on Principals of Biosafety in the BMBL.

3.2 Laboratory Practices and Techniques

Strict adherence to standard microbiological practices and techniques is essential for successful containment. Most exposures and subsequent infections occur while performing routine procedures and techniques.

Every manipulation of a biological sample has the potential for releasing a portion of the sample in microdroplet form to the air and work surfaces. One way to view the potential for release of biological agents from a given sample is to consider the amount of energy that is used to manipulate the sample. High-energy techniques (i.e. homogenization) have the potential to release aerosols of the sample if not properly contained. However, even low energy procedures such as removing screw caps and pouring or stirring of liquid medium can release aerosols of the sample. Other examples of procedures that can generate aerosolized biohazards include:

- Washing down animal rooms
- Laboratory dishwashing
- Transferring tissue culture media
- Centrifugation
- Separating blood serum

Aerosols have the potential to contaminate work surfaces, exposed skin and garments, and air in the breathing zone. Therefore, aerosols can result in topical, oral, and respiratory exposures for workers. The results of one study investigating the formation of aerosols during common laboratory procedures are shown in the table below. It should be noted that some of the

selected procedures involve the use of animals. These findings emphasize the importance of adhering to standard microbiological techniques and containment.

Table 1: Aerosols Created by Common Laboratory Procedures

Technique	Average Colonies Recovered from Air During Operation
Pipetting 10 mL culture into 1,000 mL broth	2.4
Drop of culture falling 12 in. onto Stainless steel	49.0
Painted wood	43.0
Hand towel with 5% phenol	4.0
Re-suspending centrifuged cells with pipette	4.5
Blowing out last drop from pipette	3.8
Shattering tube during centrifuging	1183.0
Inserting hot loop into broth culture	8.7
Streaking agar plates	0.2
Withdrawing syringe and needle from vaccine bottle	16.0
Injecting 10 guinea pigs	16.0
Making dilutions with syringe and needle	2.3
Using syringe/needle for intranasal inoculation of mice	27.0
Harvesting allantoic fluid from 5 eggs	5.6

Personal hygiene practices provide the simplest yet most important means for preventing disease transmission. This is especially true for workers who directly handle animals or animal tissues/body fluids. Practices such as routine hand washing at each available opportunity can be very successful in preventing contamination of more susceptible regions of the body, as well as inanimate surfaces.

Specifics on standard microbiological practices and techniques are discussed in more detail in the “Standard Biosafety Practices” section in the BMBL and in "Prudent Practices in the Laboratory – Handling and Disposal of Chemicals". Development of, and adherence to, standard microbiological practices is fundamental to the practice of biosafety. Safety equipment and laboratory design cannot be counted on to compensate for a lack of these practices.

3.3 Safety Equipment

Safety equipment includes safety centrifuge cups, biological safety cabinet (BSC's) and enclosed containers. Safety equipment also includes personal protective equipment (PPE) such as gloves, lab coats or gowns, respirators, safety glasses and goggles. Safety equipment is often referred to as a primary barrier, since it generally represents the initial barrier(s) of protections downstream from the potential hazard.

Combinations of various types of safety equipment can be used to create more than one primary barrier. However, circumstances may make it impractical to use equipment such as BSC's or completely enclosed containers, leaving PPE as the only primary barrier between the worker and a sample containing an infectious agent. This again illustrates the importance of standard microbiological practices because of the potential for PPE or other safety equipment failure. The use of safety equipment is discussed further in the BMBL.

3.4 Facility Design

The design of a facility used to conduct research involving specific biological agents is highly dependent on the epidemiology and the risk and route of transmission associated with those agents. Facility design is viewed as a secondary barrier to protect workers, both inside and outside the facility. These secondary barriers may include separation of the laboratory work area from public access, hand washing facilities, specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, or controlled or restricted access zones. More information on design criteria for specific agents and biosafety levels is found in the BMBL.

As risk of transmission increases, the number of requirements for facility design also increases. Evaluation of risk associated with a given human pathogen is a highly subjective task. The epidemiology and etiology associated with a specific human pathogen may be a steadily evolving course of events. Thus, facility design should not be viewed as a substitute for standard microbiological practices. To minimize risk of transmission, the first aspect to consider is engineering controls, followed by administrative controls. The last route of protections should be wearing of PPE.

3.5 Risk Assessment

Risk assessment is a process used to examine the various factors associated with a procedure involving biological materials in order to identify the hazardous characteristics of the material, the activities that can result in an exposure to an infectious agent, the likelihood that exposure will cause a laboratory acquired infection, and the probable consequences of an infection. The information identified by risk assessment will provide a guide for the selection of biosafety levels, microbiological practices, safety equipment, and facility safeguards that can prevent laboratory acquired infections and reduce the risk of environmental contamination. Factors to consider in a risk assessment include both agent hazards and laboratory procedure factors.

3.5.1 Agent Hazards:

1. Capability to infect and cause disease in a susceptible host
2. Virulence as measured by the severity of disease
3. Availability of preventive measures and effective treatments for the disease
4. Probable routes of transmission of laboratory infection:

a) mucous membrane exposure
b) parenteral injection
c) ingestion
d) inhalation
e) dermal

5. Infective dose
6. Stability in the environment
7. Host range
8. Its endemic nature
9. Confirmed reports of laboratory acquired infections
10. Origin of the agent

3.5.2 Hazardous Characteristics of Laboratory Procedures:

1. Procedures and operations that generate aerosols
2. Agent concentration and suspension volume
3. Use of sharps
4. Procedures that involve animals
    a) Bites and scratches
    b) Exposure to zoonotic agents
5. Complexity of a laboratory procedure

3.5.3 Potential Hazards Associated with Work Practices, Safety Equipment and Facility Safeguards:

1. Potential deficiencies in laboratory worker training and proficiency
2. Inadequate training in the selection and use of personal protective equipment
3. Safety equipment that does not work properly
4. Inadequate training on the proper use and operation of safety equipment
5. Loss of directional airflow and integrity of the facility's HVAC system

Biological risk assessment is a subjective process that requires careful consideration of the potential hazards associated with the biological agents, laboratory procedures, and the facility itself. The Centers for Disease Control and Prevention publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) describes a five step approach to provide structure to the risk assessment process.

1. Identify hazards associated with the agent and perform an initial assessment of risk.
2. Identify laboratory procedure hazards.
3. Make a determination of the appropriate biosafety level and incorporate additional precautions indicated by the risk assessment. (determination of appropriate biosafety level should be done in consultation with biosafety professional)
4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.
5. Review the risk assessment with a biosafety professional, subject matter expert and the Institutional Biosafety Committee (IBC).

Any new knowledge and experience may justify re-examining the risk assessment and the safeguards that were put in place. Risk assessment must be the basis for any recommended change.

4 The University of Colorado Colorado Springs Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) is responsible for reviewing all University research and teaching activities involving the use of biohazards, recombinant or synthetic nucleic acid molecules, select agents, or bloodborne pathogens whether the activities are carried out on campus or off campus (usually under other Institutional Biosafety Committees at other institutions).

Most biological research requires IBC authorization prior to initiation. This authorization must be renewed every 3 years.

The IBC meets regularly and will review and authorize research involving: any biological agents, infected animals or tissues (including fieldwork), recombinant or synthetic nucleic acid molecules, select agents and toxins, and work with human blood, bodily fluids, tissues or cells in culture.

4.1 Biosafety Application

Researchers can complete the IBC Biosafety application and submit it to EHS for pre-review. If there are corrections to be made or if the application needs to have more information added for clarification, the application will be returned to the researcher for modification. The completed biosafety application is then sent to a designated member for review and presentation during the next scheduled IBC meeting. Researchers are notified of the results of the IBC review. Once the IBC Biosafety application has been approved and all of the personnel listed on the protocol have successfully completed the appropriate training, the letter of approval will be sent to the Principal Investigator.

4.2 Recombinant or Synthetic Nucleic Acid Molecule Research

As a condition for funding of recombinant or synthetic nucleic acid molecule research, UCCS must ensure that research conducted at or sponsored by UCCS, irrespective of the source of funding, complies with the most current National Institutes of Health (NIH) *Guidelines for Research Involving* Recombinant *or Synthetic Nucleic Acid Molecules* *https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf*. At UCCS, the responsibility for ensuring that recombinant or synthetic nucleic acid molecule activities comply with all applicable guidelines rests with the institution and the Institutional Biosafety Committee (IBC) acting on its behalf.

Before experiments involving recombinant or synthetic nucleic acid molecule research can begin at UCCS, the Principal Investigator (PI) must submit an IBC Biosafety Application. A copy of UCCS's IBC Biosafety Application can be found on the UCCS EHS website.

All recombinant DNA research proposals require the PI to make an initial determination of the required level of physical and biological containment. For that reason, the NIH has developed six categories (**III-A** to **III-F**) addressing different types of rDNA research.

If the proposed research falls within section **III-A** of the NIH Guidelines, the experiment is considered a "Major Action". This includes experiments involving human gene transfer experiments. As a result, the experiment cannot be initiated without submission of relevant information to the Office of Science Policy (OSP) at the NIH. In addition, the proposal has to be published in the Federal Register for 15 days, it needs to be reviewed by the Recombinant DNA Advisory Committee *(*RAC), and specific approval by the NIH has to be obtained. The containment conditions for such an experiment will be recommended by the RAC and set by the NIH at the time of approval. The proposal requires IBC approval before initiation.

If the proposed research falls within section **III-B**, the research cannot be initiated without submission of relevant information on the proposed experiment to NIH/OSP (For exceptions see the NIH guidelines). Experiments covered in III-B include the cloning of toxic molecules. The containment conditions for such experiments will be determined by NIH/OSP in consultation with *ad hoc* experts. Such experiments require Institutional Biosafety Committee approval before initiation. Please refer to the guidelines for more specifics.

In section **III-C**, experiments with human subjects are covered. These experiments require IBC and IRB (Institutional Review Board) approval and NIH/OSP registration before initiation.

Section **III-D,** the next category, covers whole animal or plant experiments as well as projects involving DNA from Risk Group 2, 3 or 4 agents. Prior to the initiation of an experiment that falls into Section **III-D**, the PI must submit an IBC Biosafety Application to the Institutional Biosafety Committee. The IBC reviews and approves all experiments in this category prior to their initiation.

Section **III-E** experiments require that the filing of an IBC Biosafety Application with the IBC at the time the experiment is initiated. The IBC reviews and approves all such proposals, but IBC review and approval prior to initiation of the experiment is not required.

Section **III-F** experiments are exempt from the NIH Guidelines but a registration with the UCCS IBC is still required.

For much more detailed and thorough information on the requirements for conducting research involving recombinant or synthetic nucleic acid molecules please refer to the National Institutes of Health (NIH) *Guidelines for Research Involving* Recombinan*t or Synthetic Nucleic Acid Molecules* *https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf.*

4.3 Reporting Requirements for Incidents Involving Recombinant or Synthetic Nucleic Acids, Violations of the NIH Guidelines, or other Significant Research Related Accidents

The NIH *Guidelines for Research Involving* Recombinant *or Synthetic Nucleic Acid Molecules* states that any significant problems, violations of the NIH Guidelines, or any significant research related accidents, exposures and/or illnesses must be reported to the NIH Office of Biotechnology Activities (OBA) within **30 days.** Certain types of incidents must be reported sooner. **Spills or accidents in BSL-2 laboratories that result in an overt exposure must be reported to NIH OBA immediately**.

What Types of Incidents Must be Reported to NIH OBA?

Any spill or accident involving recombinant or synthetic nucleic acid molecules that occurs in BSL-2 laboratories or higher, leads to a personal injury or illness, or results in a breach of containment must be reported to NIH OBA. Examples of such incidents are:

1. Skin punctures with needles containing recombinant or synthetic nucleic acid molecules
2. The escape or improper disposition of a transgenic animal
3. Spills of high-risk recombinant or synthetic nucleic acids outside of a biosafety cabinet
4. Failure to adhere to containment requirements and appropriate biosafety practices as outlined in the NIH Guidelines must be reported to NIH OBA.

Minor spills of low risk agents that do not involve a breach of containment and were properly decontaminated and disposed of normally do no need to be reported.

If there is any doubt about whether an incident should be reported please contact the **Environmental Health and Safety 719-255-3212**. NIH OBA should be consulted if the IBC, investigator, or other institutional staff are uncertain whether an incident should be reported.

4.3.1 Reporting Procedure at UCCS

1. Incidents that occur at UCCS that involve recombinant or synthetic nucleic acid molecules, incidents that result in an overt exposure to materials containing recombinant or synthetic nucleic acids or any risk group 2 agent in a BSL-2/.ABSL-2 laboratory must be reported to the **UCCS EHS at 719-255-3212**
2. The Biosafety Officer will work with the Primary Investigator to gather the details of the incident to make a determination if the incident does need to be reported to NIH OBA, and if deemed necessary, consult with NIH OBA to determine if the incident warrants a report.
3. If a report is deemed necessary, the Biosafety Officer will work with the Primary investigator to complete the report. The report should contain sufficient information to explain the nature and consequences of the incident as well as the cause. The report should also include the measures that were taken to mitigate the problem and to prevent a similar incident from happening again. An incident reporting template and additional information is available from NIH OBA to facilitate the reporting process.
4. The Biosafety Officer shall inform the IBC and Institutional Official of the incident and provide a copy of the report for review.
5. NIH OBA may require other information be provided such as

a. A copy of the IBC meeting minutes documenting approval of the relevant protocol for the research laboratory in which the incident occurred.
b. A copy of the IBC minutes documenting that the incident was reviewed.
c. Policies that were in place at the time the incident occurred.
d. Revised policies or procedures that were prepared in response to the incident.

6. Training records for the personnel who were involved in the incident.
7. The Biosafety Officer shall submit the written report to NIH OBA.

5 Biosafety Lab Inspections

Every researcher who submits an IBC Biosafety Application must also have a Biosafety Lab Inspection/Audit completed. The Biosafety Lab inspections/audits are conducted by EHS. The Biosafety Lab Inspection process addresses several key laboratory safety issues including contamination control, inventory control, biosafety training, engineering controls, administrative controls, containment and other pertinent elements of laboratory safety. A copy of the Laboratory Biosafety Checklist is available at https://ehs.uccs.edu/hazardous-materials-management/biosafety. Biosafety Lab inspections are generally conducted on an annual basis.

6 Bloodborne Pathogens

UCCS has adopted the Occupational Safety and Health Administration (OSHA) 1910.1030 OSHA Bloodborne Pathogen Standard to protect workers who may be exposed to blood from microorganisms that can cause disease in humans. Such pathogens include the hepatitis B virus (HBV), hepatitis C virus and the human immunodeficiency virus (HIV).

6.1 Work Involving Risk Group 2 Agents

In any laboratory where work involves the use of and/or exposure to Risk 2 agents such as human blood, blood products, reagents derived from blood, human cell lines, unfixed human tissue, non-human primate cell lines, or other materials that are known or reasonably likely to contain or be infected with HIV, Hepatitis B virus, Hepatitis C virus or that could support the replication of HIV (e.g. HeLa, Hek 293, etc.), or field work that involves the potential for exposure to infectious agents such as plague, hantavirus, rabies, etc., must complete a Biohazard Control Plan as a part of their IBC Biosafety Application. The Biohazard Control Plan addresses important elements such as exposure determination, controls methods, laboratory/facility cleaning and decontamination, safety devices and containment used, personal protective equipment, vaccinations, accident procedures, procedures for waste disposal, and training. Copies of examples of exposure controls plans for Human Blood, Body Fluids, and Reagents Derived from Blood or Body Fluids; human cell lines (e.g. HeLa, HEK 293, etc.) can be found at the following links: Please read carefully and make sure that the “Biohazard Control Plan” that will be submitted to the IBC for review and approval is specific to your proposed research and the associated biohazards.

6.2 Select Agents

Infectious agents and toxins that are considered by the Department of Health and Human Services (DHHS) or the United States Department of Agriculture (USDA) as having the potential to pose substantial harm or a severe threat to human, animal, or plant health or plant products are regulated as “select agents”.

Select agents in any quantity are **not permissible** at UCCS. Toxins are permissible when in exempt quantities only. If you would like to work with toxins in exempt quantities, please contact the EHS. Information regarding the Federal Select Agent Program can be found at:

- Select Agent Website: http://www.selectagents.gov/
- Select Agents and Toxins List: http://www.selectagents.gov/SelectAgentsandToxinsList.html
- Permissible Toxin Amounts: http://www.selectagents.gov/PermissibleToxinAmounts.html

6.3 Lentiviral Vectors

The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety concerns. The NIH Office of Biotechnology Activities (now the Office of Science Policy) received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting, the RAC offered the following findings and recommendations: https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf. For assistance with performing a risk assessment for work with lentiviral vectors and other viral vectors please contact EHS.

7 Biosafety Risk Levels

Table 2 Biosafety levels

The table below provides a brief summary of the four biosafety levels, relative health risk associated with each level and some examples of microorganisms classified at each level.

BSL	Description	Health Risk	Examples
1	Not known to consistently cause disease in healthy adults	Low individual risk, low community risk	• E. coli K12 • *Bacillus subtilis*

2	Associated with human disease. Treatment and/or vaccine are *often* available	Moderate individual risk, low community risk	• *Salmonella* sp. • MRSA • *E. coli* O157 • Human, non-human primate bodily tissues or fluids, including cell lines
3*	Agents associated with serious or lethal human disease. Preventative or therapeutic interventions may be available	High individual risk, low community risk	• *Yersinia pestis* • *Mycobacterium tuberculosis*
4*	Agents likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available	High individual risk, high community risk	• Ebola • Marburg Virus

***Agents requiring BSL-3 and BSL-4 facilities and practices are not permitted at UCCS**.

8 Biosafety Level 1 (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent 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 appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

8.1 Standard Microbiological Practices

The laboratory supervisor must enforce the departmental policies that control access to the laboratory.

a) Persons must wash their hands after working with all potentially hazardous materials including recombinant or synthetic nucleic acids, potentially infectious materials, chemicals etc., before leaving the laboratory.
b) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

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

8.1.1 Sharps Management

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

a) 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.
b) Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
c) Non disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
d) 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.

8.1.2 Splashes or Aerosol creation

Perform all procedures to minimize the creation of splashes and/or aerosols.

a) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material or recombinant or synthetic nucleic acids with appropriate disinfectant. Work surfaces are decontaminated at least once a day for all recombinant or synthetic nucleic acids work.
b) Place a physical barrier between the spill and yourself to contain aerosols. Generally, a paper towel or towels will be sufficient for this purpose.
c) Pour, or spray **appropriate disinfectant** on the paper towel.
d) Leave for appropriate contact period to inactivate the spilled material.
e) Clean up after the inactivation period. Dispose of all clean up materials in the biohazardous waste stream.
f) For biological agent spills inform both the lab director and EHS.

8.1.3 Decontamination

a) Decontaminate all cultures, stocks, recombinant or synthetic nucleic acids and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    i. Materials to be decontaminated outside of the immediate laboratory 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.
b) All spills and accidents involving potentially biohazardous material are reported to the laboratory director and to the Biosafety Officer as soon as possible. This reporting is important to safeguard personnel at UCCS who work with potentially biohazardous material and facilitate compliance with application local, state and federal regulations and other legal obligations.

c) Equipment and pertinent lab areas are cleaned and decontaminated before workers, (Facility Maintenance, Vendors, Contractors or other non-lab workers) are asked to move equipment or work in the lab area. Equipment decontamination follows the manufacturer's recommendations and is effective for the potentially biohazardous materials that are in use with the piece of equipment.
d) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory. The sign should include the name and phone number of the lab director or other responsible personnel.
e) An insect and rodent control program is in effect. The laboratory is routinely inspected for evidence or signs of infestation. If found the Laboratory Director is immediately notified.
f) The laboratory director must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposure, 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 having these conditions are advised to self-identify to their own health care provider for appropriate counseling and guidance.

8.2 Safety Equipment (Primary Barriers)

a) Protective laboratory coats, gowns or uniforms are recommended to prevent contamination of personal clothing.
b) Wear protective eyewear 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. Glove selection should be based on an appropriate risk assessment. Alternative to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition:
    i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
    ii. Remove gloves and wash hands when work with hazardous material has been completed and before leaving the laboratory.
    iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste (biohazardous waste stream). Hand washing protocols must be rigorously followed.
    iv. Gloves are removed before leaving the laboratory and before touching common use items such as telephones, doorknobs, keyboards, drawer handles etc.

8.3 Laboratory Facilities (Secondary Barriers)

a) Laboratories should have doors for access control.
b) Each laboratory 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 appropriate.

d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals.
   ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant(s).
e) Laboratory windows that open to the exterior should be fitted with screens.

9 Biosafety Level 2 (BSL-2)

Biosafety level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.
- Access to the laboratory is restricted when work is being conducted; and
- All procedures in which infectious aerosols or splashes may be created are conducted in BSC's or other physical containment equipment.

9.1 Standard Microbiological Practices

a) Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents or recombinant or synthetic nucleic acid molecules is in progress. Additionally, the PI must enforce institutional policies that control access to the laboratory. The door should be kept closed whenever work is being performed with Risk Group 2 agents in the laboratory.
b) Persons must wash their hands after working with potentially hazardous materials, recombinant or synthetic nucleic acid molecules, potentially infectious materials, chemicals, etc., Gloves must be removed prior to leaving the laboratory.
c) Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human consumption is not permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
d) Contact lens users should wear safety glasses, goggles or face shields.
e) Mouth pipetting is prohibited; mechanical pipetting devices must be used.
f) 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.

9.1.1 Sharps Management

Precautions including those listed below must always be taken with sharp items. These include:

a) 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.
b) Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
c) Extreme caution is used when handling needles and syringes to avoid autoinoculation and the generation of aerosol during use and disposal.
d) Syringes which re-sheath the needle, needle-less systems, and other safety devices should be used. If these are not practical, please consult with EH&S to ensure that you have the appropriate documentation required for your current needle use practices.
e) Broken glass 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.

9.1.2 Splashes or Aerosol creation

Perform all procedures to minimize the creation of splashes and/or aerosols.

a) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material or recombinant or synthetic nucleic acids with appropriate disinfectant. Work surfaces are decontaminated at least once a day for all recombinant or synthetic nucleic acids work.
b) Place a physical barrier between the spill and yourself to contain aerosols. Generally, a paper towel or towels will be sufficient for this purpose.
c) Pour, or spray **appropriate disinfectant** on the paper towel.
d) Leave for appropriate contact period to inactivate the spilled material.
e) Clean up after the inactivation period. Dispose of all clean up materials in the biohazardous waste stream.
f) For biological agent spills inform both the lab director and EHS.

9.1.3 Decontamination

a) Decontaminate work surfaces daily after completion of work and after any spill or splash of potentially infectious material or recombinant or synthetic nucleic acid molecules with appropriate disinfectant.
b) Decontaminate all cultures, stocks, recombinant or synthetic nucleic acid molecules and other potentially infectious materials before disposal using an effective method.
    i. Materials to be decontaminated outside of the immediate laboratory 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.
c) All infectious liquid or solid wastes are decontaminated before disposal. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in durable, labeled, leak-proof containers which are sealed and labeled appropriately before being removed from the laboratory.
d) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the

laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information for BSL-2 labs is posted on this sign also in accordance with institutional policy.

e) An insect and rodent control program is in effect. The laboratory is routinely inspected for evidence or signs of infestation. If found the Laboratory Director is immediately notified.

g) The laboratory supervisor must ensure 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 having these conditions are advised to self-identify to their own health care provider for appropriate counseling and guidance.

9.2 Special Practices

All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

a) Personnel are advised of special hazards and are required to read instructions on practices and procedures, and to follow them.

b) Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
   i. 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

c) The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

d) Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

e) Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
   i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
   ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

f) 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 supervisor. Medical evaluation, surveillance, and treatment should be provided, and appropriate records maintained.

i. **All accidents resulting in overt or potential exposure are immediately reported to the Biosafety Officer and /or the Institutional Biosafety Committee.**

g) Animals and plants not associated with the work being performed must not be permitted in the laboratory.

h) All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

i) Laboratory 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. Special care is taken to avoid skin contamination with infectious materials and with organisms containing recombinant or synthetic nucleic acid molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

9.3 Safety Equipment (Primary Barriers)

a) Properly maintained Biosafety Cabinets (BSC's) (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

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

ii. High concentration or large volumes of infectious agents are used. 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. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

c) Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

d) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternative to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:

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

ii. Remove gloves and wash hands when work with hazardous material has been completed and before leaving the laboratory.
iii. 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 should be used in rooms containing infected animals as determined by the risk assessment.

9.3.1 Laboratory Facilities (*Secondary Barriers) &* Personal Protective Equipment

a) Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
b) 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.
c) The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectants.
e) Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, the must be fitted with fly screens.
f) BSC's must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSC's should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
g) Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
h) An eyewash station must be readily available.
i) 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.
j) HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. Provisions to assure proper safety cabinet performance and air system operation must be verified.
k) A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
l) A written assessment of laboratory risks must be performed to determine the appropriate personal protective equipment (PPE) required for a particular task. The appropriate PPE will be assigned for each potentially hazardous task performed in the lab. Include decontamination and waste disposal processes.

10 Animal Biosafety

Currently UCCS does not conduct any research involving animals subject to Animal Biosafety requirements.

11 Diagnostic Work- What to do if you Culture a BSL-3 Organism

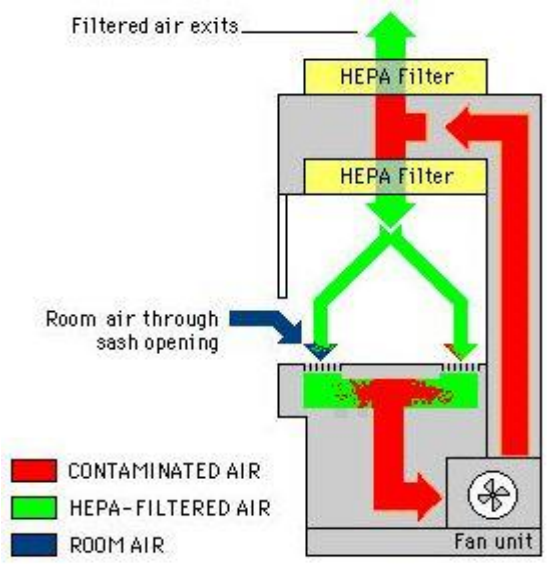
If during the course of conducting diagnostic work in the laboratory a BSL-3 organism or select agent or toxin is identified you must stop work with that material, secure it against theft, loss, or release, and call EHS immediately so that the appropriate regulatory agencies can be notified. UCCS is **not** registered for the possession, use and transfer of select agents and toxins.

12 Biosafety Cabinets

12.1 What is a Biosafety Cabinet?

A biosafety cabinet (BSC) is **not** a chemical fume hood. Chemical fume hoods are designed to protect personnel by removing chemical vapors and aerosols away from the work area. BSCs are designed to protect personnel, the products being handled, and the environment from particulate hazards, such as aerosolized infectious microorganisms. BSCs use uniform vertical laminar airflow to create a barrier to airborne particulates. BSCs utilize High Efficiency Particulate Air (HEPA) filters to clean both the air entering the work area and the air exhausted to the environment.

The HEPA filter removes airborne particles from the air but does not remove chemical fumes. Only biosafety cabinets that are exhausted via duct work are appropriate for use with small amounts of toxic volatile chemicals. Always use a fume hood when working with large amounts of toxic volatile chemicals. Appendix A of the 5th edition of the BMBL titled “Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets” provides more detailed information on the different types of BSCs: BMBL Appendix A



Class II Biological Safety Cabinet

http://www.ars.usda.gov/News/docs.htm?docid=14605&page=3

12.2 When Must I Use a BSC?

Biosafety cabinets should be used whenever you are conducting lab procedures with biohazardous materials that may produce aerosols, or anytime you are working with large amounts of infectious materials. BSCs, when properly used, have been shown to be highly effective in reducing laboratory-acquired infections and cross-contamination of cultures due to aerosol exposures. BSCs also protect the environment. BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets designated as Class I, II, and III are available. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems. The HEPA filter traps 99.97% of particles of 0.3 µm in diameter and 99.99% of particles of greater or smaller size. Biological safety cabinets must not be confused with other laminar flow devices or "clean benches." Horizontal flow cabinets direct air towards the operator and should never be used for handling infectious or toxic materials.

For additional information on biological safety cabinets, please visit: NIH-CDC, Appendix A, Biosafety in Microbiological and Biomedical Laboratories

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 B1and 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

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	V o l
I	75	In at front through HEPA to the outside or into the room through HEPA	Yes	W h
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into	Yes, minute amounts	No
II, B1	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a	Yes	Y e s
II, B2	100	No recirculation; total exhaust to the outside through a HEPA filter	Yes	Yes, small
II, A2	100	Similar to II, A1, but has 100 fpm intake air velocity and plenums are under negative pressure to room; exhaust air can be	Yes	W he n ex
III	N/A	Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside	Yes	Yes, small amount

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 component 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.

12.3 Open Flames in a BSC

Open flames, such as Bunsen burners, should never be used in a BSC. Open flames inside of a BSC disrupt the airflow, compromising protection of both the worker and the material being handled. Open flames are extremely dangerous around flammable materials, such as ethanol, which is often found in a BSC. Electric incinerators or sterile disposable instruments are excellent alternatives.

12.4 Decontamination and Ultraviolet Lights in a BSC

The BSC work area must always be cleaned and disinfected thoroughly before and after each use, using a chemical disinfectant such as an iodophor. Be sure to allow adequate disinfection time for the disinfectant used. 70% alcohol can evaporate too quickly to be effective and fumes can build up in the biosafety cabinet, creating a potential explosion hazard. If you use bleach as a disinfectant, be sure to follow by wiping with sterile water, as bleach will corrode the stainless steel of the biosafety cabinet. The use of ultraviolet (UV) lights in a biosafety cabinet is not recommended because of their ineffectiveness and safety risk. UV light has very little power to penetrate, even though a dust particle, so the UV light is not a method that should be used for primary decontamination. Note that UV lights lose effectiveness over time. Warning: Be sure the UV light is turned off before beginning work. Exposure to UV light for a prolonged period will cause skin, corneal and/or retinal burns. Newer BSCs have safeguards to prevent personnel from being exposed to UV light; however, some older models may not have these safeguards. For most consistent contamination control and safe operation, biosafety cabinets should be run 24 hours a day, 7 days a week.

12.5 Annual Certification Testing

Per NIH and CDC, all BSCs are to be field certified to the current NSF/ANSI Standard 49, Annex F upon installation, and annually thereafter, and whenever they are moved or relocated. Environmental Health and Safety strongly recommends using vendor technicians who are accredited by NSF-49, and have been trained to perform field certifications to NSF/ANSI Standard 49, Annex F. The vendor must be able to provide documentation/verification of their technician's current NSF-49 accreditation. The standard used to properly evaluate Class II biosafety cabinets, NSF/ANSI Standard 49 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification, is designed to minimize hazards inherent in work with agents assigned to Biosafety Levels or Animal Biosafety Levels 1, 2, or 3 at this institution. It also defines the tests that cabinets must pass to meet this Standard.

Below is the list of current (as of June 2019) vendors approved to perform work on Biological Safety Cabinets on this campus. These vendors have provided the current certifications to meet national best practices and NSF standards.

Technical Safety Services (TSS) Andrea Lauritzen 720-981-4965 | alauritzen@techsafety.com

Sercom Serena Laubscher 970-482-8410 | info@sercom-usa.com

ENV Services Caitlin Des Rosier 210-690-3368, ext. 1282 Cell: 210-279-8890 | cdesrosier@envservices.com

HSS Stephanie Polk 303-603-3060 | stephanie.polk@hss-us.com

If you have another vendor that you would like to utilize, please contact EHS.

12.6 Moving or Repairs

Filter changes and repairs must be done by the contracted qualified servicing company. This company will also be responsible for filter disposal.

BSCs must be recertified whenever they are moved or have the filters changed. If you planning to move your BSC please contact a qualified servicing company prior to the move. Once the BSC has reached its new location it will have to be re-certified by a qualified servicing company before it is used. For questions related to moving a BSC or for a list of NSF certified service providers in the area, please contact the Environmental Health and Safety office.

12.7 Purchasing and Installing a New BSC

If plans exist for the purchase of a new BSC, the Environmental Health and Safety Office must be notified to provide assistance in choosing the appropriate BSC and for ensuring that the BSC is put on the annual certification testing schedule.

13 Spill Response

Response to Spills and Exposures Involving Biological Materials and Recombinant or Synthetic Nucleic Acid Molecules

In the event of accidental spills or exposures, the first priority should be the safety and welfare of facility personnel. The second priority should be containment of the exposure. In all cases, care should be taken to avoid tracking spills through the facility and broadening the exposure.

General: Biohazards include body fluids, blood, infectious waste, recombinant or synthetic nucleic acid molecules, or potentially infectious material. Any body fluid may contain microorganisms capable of causing disease. Therefore, appropriate protective attire must be worn when having direct contact with any type of body fluid or tissue. Gloves must be changed, and hands washed after handling laboratory specimens containing body fluids and between animal examinations. All procedures involving blood or other potentially infectious materials must be performed in a manner that minimizes splashing, spraying, and aerosolization of these substances.

13.1 Personal Readiness Activities

- Provide immediate first aid.
- Eyes or mouth splattered with blood, biological organisms, recombinant or synthetic nucleic molecules, or body fluid:
    - Flush with water at least 15 minutes.
    - Use the eyewash stations located in the facility/lab.
- Needle stick
    - Milk wound to induce bleeding.
    - Wash with soap and water for at least 15 minutes.
- All injuries
    - Remove contaminated clothing, wash skin, and replace with clean clothing.
    - Get medical attention/consultation for exposures to biohazardous materials

- Call 911 for medical emergencies
- Contact the Biosafety Officer at 719-255-3212 or after hours, UCCS Police 719-255-3111to contact EHS.
- Submit an Accident/Illness Report Form online https://surveyuccs.co1.qualtrics.com/jfe/form/SV_0Tk4mb7gJCTRSnj

13.2 Cleanup Actions: Small Spill

- Protect body by putting on protective clothing (gloves, eye protection, and lab coat).
- Provide first aid if needed.
- Cover the spill with paper towels or other absorbent materials. Carefully pour the appropriate disinfectant around the edges of the spill and then work your way into the center. Allow a 20-minute contact period. Use paper towels to clean up the spill, working from the edges to the center.
- Clean spill area with fresh towels soaked in disinfectant.
- Remove broken glassware with forceps, tongs or broom and dustpan and dispose in sharps container. Do not pick up any contaminated sharp objects with your hands.
- Wipe down all equipment and surfaces that were potentially contaminated.
- Dispose of contaminated material as biohazardous waste.
- Remove all PPE before leaving area of the spill, put in a biohazard bag, and wash hands.

13.3 Cleanup Actions: Large Spill

- Evacuate the immediate area of all personnel and close the door(s). Post a person by the area to prevent re-entry. Wait 30 minutes for aerosols to settle.
- Check for exposure and provide first aid if needed.
- Follow instructions listed above for a small spill.
- Inform all personnel and lab supervisor about the spill and successful cleanup as soon as possible.
- Notify the biosafety group in the event of a spill (719-255-3212) so that all pertinent information is collected. The biosafety group will recommend a course of action based on this information.

13.4 Cleanup Actions: Spill Inside of a Biosafety Cabinet

- Wear appropriate protective clothing before proceeding with the clean-up.
- Allow the cabinet to run while addressing the spill
- Cover the spill with paper towels or other absorbent material, then carefully pour the disinfectant on surface of the towel and work your way to the center of the spill. Make sure to saturate the towel and allow to soak for a minimum of 20 minutes contact time directly on the spill.
- Wipe the walls, work surfaces, inside of sash and any potentially contaminated equipment with disinfectant soaked towels before removing it from the BSC.
- Lift exhaust grill and tray and wipe all surfaces.
- Discard contaminated disposable material using appropriate biohazardous waste disposal procedures.
- Wipe down contaminated reusable items with disinfectant then place in autoclave bag or autoclave pans with lids for autoclaving.
- Items that are non-autoclavable should be wiped down with disinfectant and kept wet for

a minimum of 20 minutes before removal from BSC.
- Remove protective clothing, when done and place in biohazard bag for disposal or autoclaving for reusable items.
- Run the BSC for at least 15 minutes after clean-up before reusing
- WASH HANDS!

13.5 Reporting Requirements

- As mentioned previously in this section, Contact the Biosafety Officer at 719-255-3212 or after hours, UCCS Police (719-255-3111) to contact EHS.
- Submit an Accident/Illness Report Form online (https://surveyuccs.co1.qualtrics.com/jfe/form/SV_0Tk4mb7gJCTRSnj).
- For incidents involving recombinant or synthetic nucleic acids please refer to the information under the section titled **"Reporting Requirements for Incidents Involving Recombinant or Synthetic Nucleic Acids, Violations of the NIH Guidelines, or other Significant Research Related Accidents".**

14 Disposal of Biohazardous Waste

Biohazardous waste is biological, infectious, and some non-infectious waste. Biological waste includes cultures, plates, media, and other materials that contain or come in contact with living cells, body fluids, viruses, clinical materials, and other microorganisms. Infectious waste is biological waste that involves the presence of organisms containing recombinant or synthetic nucleic acid molecules, or other organisms hazardous to human health. Non-infectious waste includes all examples listed under biological waste that do not meet the criteria of infectious or have been rendered non-infectious by chemical disinfection or autoclaving.

Before biohazardous waste can disposed it must be rendered non-infectious by using effective chemical disinfection methods or by autoclaving. **If in doubt, be conservative and autoclave or chemically treat all non-radioactive biological waste. Do NOT use an autoclave if your waste contains radioactive material.** If you have questions, contact the Biosafety Group.

Refer to the Biological Waste Management Plan (https://ehs.uccs.edu/waste-management/bio-waste-management)

15 Transporting Biological Materials on Campus

The following procedure for preparing and transporting biological materials between university buildings should be used:

1. Use primary containers that are designed to contain the material to be stored.
2. Place primary sample containers into an appropriate secondary container for transport. If sample material is liquid or may release liquids, use a leakproof secondary container

with a secure lid (i.e. cooler with a latch able lid). Additionally, place enough absorbent material (i.e. paper towels) in the secondary container to absorb all free liquids in the event that primary containers rupture or break during transport.

3. Package primary containers in the secondary container in a manner that will reduce shock, rupture, and/or breakage. Bubble wrap or similar shock-absorbing materials may also be used to minimize the potential for primary container rupture.
4. Label all secondary containers with a brief description of the contents and a contact name and phone number.

Please contact the EHS regarding the transport biological materials by vehicle between campus buildings.

16 Shipping of Biological Materials to an Off-Campus Destination

Transportation of biological materials is an activity that affects all research and diagnostic service entities. In some instances, these materials may be regulated for transportation and will require specific packaging, labeling and documentation. Additionally, the shipper must have documented training relative to his or her tasks associated with the shipment. This is the case for shipment of diagnostic specimens (from humans or animals), cultures of infectious substances (infectious to humans and/or animals), genetically modified organisms and any biological materials shipped on dry ice. In light of recent current events, there is an increased level of surveillance on the part of federal and international authorities for all hazardous materials/dangerous goods shipments that may include diagnostic specimens and infectious substances.

As a shipper, it is essential to ensure that materials are properly classified and that all applicable regulatory provisions for shipment are met. EHS offers training and consultation for campus personnel who plan to ship biological materials including diagnostic specimens, infectious substances, genetically modified organisms, and biological materials on dry ice.

Impact of non-compliance:

• Increased risk of material release during the shipping process.

• May result in refusal or return of packages during the shipping process. This could be critical if materials are temperature sensitive.

• May result in fines from the Federal Aviation Administration (FAA).

16.1 Preparing to Ship Biological Materials:

Before you package and ship materials to an off-campus destination there are several items that should be addressed.

1. You must successfully complete the online training course for Shipping Biological Materials course available on line at: https://ehs.uccs.edu/training/biosafety-training

   The purpose of this training module is to familiarize the Principal Investigator and lab personnel with the regulations, different shipping categories, and proper labeling and packaging of biological materials. This course must be taken every two years or whenever relevant regulations change.

2. The Environmental Health and Safety Office Biosafety Group can provide assistance with the shipping process.
3. There are some other important considerations involved in the shipping of biological materials such as:
   i. Materials Transfer Agreement
   ii. Export Controls and Trade Sanctions
   iii. Importing Biological Materials - Importing Biologics and Vectors: Know Before You Go

17 Security

Laboratory security is an important part of an effective safety program. Follow these steps to ensure a secure working environment in your laboratory:

1. Keep laboratory doors closed and locked when unoccupied.
2. Keep stocks of organisms and hazardous chemicals locked when the laboratory is unoccupied.
3. Keep an accurate record of chemicals, stocks, cultures, project materials, growth media, and those items that support project activities.
4. Notify Environmental Health and Safety and UCCS police if materials are damaged or missing from laboratories.
5. Inspect all packages arriving at the laboratory.
6. When research is completed for the day, ensure that chemicals and biological materials have been stored properly and securely.
7. Decontaminate materials and work surfaces after completing work and at least daily.
8. Turn off equipment, flames, steam supply, and electrical appliances after completing work.
9. Ask strangers (someone you do not recognize as a co-worker or support staff person) to exit the room if they are not authorized to be there.
10. Discuss other security-specific requirements with your supervisor.

18 Required Training

When an IBC Biosafety application is submitted, all aspects of the protocol is reviewed so that the appropriate biosafety training can be assigned to those individuals listed on the application. Individuals are notified of the training courses that they must successfully complete before final approval is granted. EHS tracks all of the training and notifies laboratory personnel when they

are due for refresher training. Required training may be accessed on the UCCS EHS website at: https://ehs.uccs.edu/training

19 Biological Sample Inventories

It's time to clean shop and create/update an inventory – do you know what's in your lab's freezers?

UCCS researchers are responsible for what is in their laboratories. Please discuss this topic with your lab members and update your lab's inventories of biological materials. It not only keeps you aware of the agents for which you are ultimately responsible, but it will also help create space in your freezers by getting rid of those tubes that you no longer need. Freezer clean-outs are good laboratory practice, and they help keep your freezers running well and efficiently, thereby decreasing the risk of freezer malfunction and sample loss. A proper inventory should also greatly reduce the amount of time needed to find a sample from the freezer.

Below are a few examples of templates that can be used for your inventory:

9×9 freezer box template
10×10 freezer box template
Bacteria stock storage sheet
Quartzy (allows you to track cell lines, freezers, etc.)

19.1 Guidance for cleaning out Low Temp Freezers

This document describes procedures to help laboratories clean out and dispose of unused or unwanted items contained in ULT freezers and other freezers and refrigerators. All items removed from any freezer or refrigerator must be carefully evaluated to ensure that they are disposed of properly. For most biological kits and biological samples there is a consolidation procedure described below that can be implemented by laboratory personnel and will greatly reduce the volume of waste and the amount of hazardous waste tags that need to be completed by the research group. For chemical reagents, EH&S has a segregation procedure that will reduce the amount of hazardous waste tags that need to be completed; this also described below.

Guidance Document for Low Temp Freezer Cleanouts

20 Biosafety Audits

We conduct Biosafety audits periodically (generally annually) to support ongoing safety. You can ensure that your lab will pass by using the checklist provided below. This audit is part of the IBC post-approval monitoring.

Biosafety Audit Checklist