CHAPTER IV: BIOHAZARD CONTAINMENT

Although the most important aspect of biohazard control is the awareness and care used by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can prevent dissemination of pathogens should their accidental release occur.

A. BIOSAFETY LEVELS

Biosafety Levels consist of combinations of laboratory practices and procedures, safety equipment, and laboratory facility design features appropriate for the operations to be performed within the lab, and are based on the potential hazards imposed by the agents used and for the specific lab activity. It is the combination of practice, equipment, and facility that form the basis for physical containment strategies for infectious agents. There are four biosafety levels, with Biosafety Level 1 (BSL-1 or BL-1) being the least stringent and Biosafety Level 4 (BSL-4 or BL4) being the most stringent. In general, BSL-1 is recommended for work with nonpathogenic microorganisms, BSL-2 is recommended for disease agents transmitted by direct contact (percutaneous inoculation, ingestion, or mucous membrane exposure), BSL-3 is recommended for disease agents with a potential for aerosol transmission, and BSL-4 is recommended when total separation between the infectious agent and investigator is critical. Risk Group designations often, but not always, correlate directly with the biosafety level appropriate for a given research activity. For example, deleting the virulence factor of a RG3 pathogen may render it safe to be handled with BSL2 facility and practices. Conversely, insertion of toxin-producing genes in an RG1 microorganism may require BSL-2 facility and practices. Furthermore, RG2 agents with potential of causing mutagenesis may require additional BSL-3 practices in a standard BSL-2 facility. The Institutional Biosafety Committee (IBC), established under the NIH Guidelines, determines the proper biosafety level for working with a particular project. One should always carefully review project-specific, approved IBC protocol prior to starting the research. This manual is designed to focus on BSL-2, but a brief description of the correlation between Risk Group and Biosafety Level and the facility design features appropriate for labs operating at the various biosafety levels is presented in Tables 1 and 2.

Table 1
RELATIONSHIP OF RISK GROUPS TO BIOSAFETY LEVELS, PRACTICES, AND EQUIPMENT

Risk Group	Biosafety Level	Laboratory Practices	Safety Equipment	Examples of Laboratories
1	Basic – BSL-1	GMT[a]	None required; open bench work	Basic teaching
2	Basic – BSL-2	GMT plus protective clothing; access control, *universal precautions* for handling sharps, biohazard sign	Open bench plus BSC[b] for activities with aerosol-potential	**Most biomedical research on the Hyde Park campus**; primary level hospital; diagnostic, teaching, and public health
3	Containment – BSL-3	As BSL-2 plus special clothing, controlled access, directional air flow	BSC and/or other primary containment for all activities	Special diagnostic; Regional Biocontainment Laboratory
4	Maximum Containment – BSL-4	As BSL-3 plus airlock entry, shower exit, special waste disposal	Class III BSC or positive pressure suits, double-ended autoclave, HEPA-filtered air	Not at University of Chicago; Dangerous pathogen units

[a] GMT, Good Microbiological Technique.
[b] BSC, Biological Safety Cabinet

Table 2
SUMMARY OF BIOSAFETY LEVEL REQUIREMENTS

	Biosafety Level			
	1	**2**	**3**	**4**
Isolation of laboratory	No	No	Desirable	Yes
Room sealable for decontamination	No	No	Desirable	Yes
Inward air flow ventilation	No	Desirable	Yes	Yes
Mechanical ventilation via building system	No	Desirable	Yes	No
Mechanical, independent ventilation	No	No	Desirable	Yes
Filtered air exhaust	No	No	Desirable	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Effluent treatment system	No	No	Desirable (BSL3-Ag)	Yes
Autoclave on site	Desirable	Yes	Yes	Yes
Autoclave in laboratory/suite	No	No	Yes	Yes
Double-ended autoclave	No	No	Desirable	Yes
Class II BSC	No	Desirable	Yes	Yes

[1]BSC, Biological Safety Cabinet

For a more comprehensive description of each of these biosafety levels, please consult the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories, 5th edition,* (2009) http://www.cdc.gov/biosafety/publications/bmbl5/.

Experiments involving recombinant DNA are also governed by another method of providing containment, namely ***biological containment***. For biological containment, highly specific biological barriers are considered in the risk assessment process. Specifically, biological containment considers natural barriers that limit either (1) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (2) its dissemination and survival in the environment. For additional information on biological containment, please consult the NIH *Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules* (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines).

B. Practices and Procedures

The following practices, **corresponding to BSL-2,** are important for the prevention of laboratory infection and disease, as well as for the reduction of the potential for contamination of experimental material. These practices and procedures provide the foundation for the more restrictive containment of RG3 organisms. If you are considering research with a RG3 organism, contact the Office of Biological Safety at 773-834-2707 for additional BSL-3 containment information.

1. Personal Hygiene

(a) Do not eat, drink, chew gum, use tobacco, apply cosmetics (including chap stick), or handle contact lenses in the laboratory.

(b) Do not store food for human consumption in laboratory refrigerators.

(c) Wash hands frequently after handling infectious materials, after removing latex/nitrile gloves and protective clothing, and always before leaving the laboratory.

(d) Keep hands away from mouth, nose, eyes, face, and hair.

(e) Do not remove personal protective equipment (such as cloth lab coats) from the lab.

(f) First-aid kit(s) should be available and not expired.

2. Laboratory Procedures for Handling Infectious Microorganisms

(a) A laboratory biosafety manual should be assembled outlining activities and defining standard operating procedures. In most cases, your lab's Institutional Biosafety Committee (IBC) protocol, together with this BSL-2 Biosafety Manual, will provide you with the necessary information to work safely.

(b) If you are working with recombinant DNA and/or working with agents at BSL-2 or higher, you must obtain approval by the UC IBC.

The IBC can be reached at 773-834-4765 or online at: http://ibc.uchicago.edu/.

(c) Principal Investigators and/or laboratory supervisors are responsible for training employees and ensuring that all personnel are informed of hazards.

(d) Plan and organize materials/equipment before starting work.

(e) Keep laboratory doors closed; limit access to lab personnel.

(f) When RG2 (or higher) pathogens are used in long-term studies, post a biohazard sign at the laboratory entrance identifying the agents in use and the appropriate emergency contact personnel. Templates of these biohazard signs will be generated by the Office of Biological Safety based upon the information provided in your lab's IBC Protocol.

(g) BSL-2 laboratories should have a sink for hand washing, an eyewash station in which the eyewash is tested/flushed weekly, be relatively clutter-free, and be easy to clean.

(h) Wear a fully fastened laboratory coat when working with infectious agents. Wear protective gloves whenever handling potentially hazardous materials, including human blood and body fluids. Wear eye protection when working in the BSL2 laboratory when necessary.

(i) Remove and leave all protective clothing, including gloves, within the laboratory before exiting. If transport of research materials through public spaces is required, one glove may be removed and ungloved hand used to handle public equipment (door handles, elevator buttons, etc.) and lab coats may be carried.

(j) Never mouth-pipette; use mechanical pipetting devices.

(k) When practical, perform all aerosol-producing procedures such as shaking, grinding, sonicating, mixing, and blending in a properly operating biological safety cabinet (BSC). Note that placement of certain equipment within the BSC may compromise cabinet function by disturbing the air curtain. BSC certification and re-certification should be performed with permanent equipment inside the BSC.

(l) Centrifuge materials containing infectious agents in durable, shatter-resistant, closable tubes. Use a centrifuge with sealed heads or screw-capped safety cups. After centrifugation, open the tubes within a BSC.

(m) Minimize the use of needles, syringes, razor blades, and other sharps when possible. After use, syringe-needle units must be disposed in a dedicated sharps container without removing or recapping the needles.

(n) Cover countertops where hazardous materials are used with plastic-backed disposable paper to absorb spills and dispose of them daily or following a spill.

(o) Wipe work surfaces with an appropriate disinfectant according to corresponding IBC protocol after experiments and immediately after spills.

(p) Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal (See Chapter V of this Manual).

(q) Report all accidents and spills to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol and the location of cleanup equipment. Step-by-step Spill response protocols should be posted in the laboratory.

(r) Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Do not forget to routinely decontaminate all shared equipment and equipment in common areas.

(s) Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate biohazard signs.
(t) Equipment used with biohazards must be decontaminated prior to repair.

C. Engineering Controls

1. Laboratory Design

The more virulent an organism, the greater the degree of physical containment required. Proper safety equipment provides primary containment; laboratory design provides secondary containment. The Office of Biological Safety is available for consultation on these matters.

2. Laboratory Ventilation

To control containment it is important that laboratory air pressure be lower than that in the adjacent spaces. This negative air pressure differential ensures that air will enter the laboratory and not egress to the hallway. While negative air pressure is recommended at BSL-2, it is required at BSL-3. **If you wish to maintain negative room pressure, laboratory doors should be kept closed while biohazardous work is taking place.**

Exhaust air from biohazardous laboratories should not be recirculated in the building. It should be ducted to the outside and released from a stack remote from the building air intake. In certain special situations, including many BSL-3 labs, air exhausting from a containment facility should be filtered through HEPA (high efficiency particulate air) filters, which can capture microorganisms.

3. Biological Safety Cabinets

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. When functioning correctly and used in conjunction with good microbiological techniques, BSCs are very effective at controlling infectious aerosols. BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed.

The following are brief descriptions of BSC types and guidelines for their use. For more in-depth descriptions, including diagrams of airflow and more detailed usage parameters, please visit this site: http://safety.uchicago.edu/pp/labsafety/biosafety/cabinets.shtml.

(a) BSC Types

Three kinds of biological safety cabinets, designated as Class I, II, and III, have been developed to meet varying research and clinical needs.

CLASS I - cabinets are manufactured on a limited basis and have largely been replaced by Class II cabinets. A Class I cabinet is essentially a HEPA-filtered chemical fume hood in which all of the air entering the cabinet is exhausted into the room or ducted to the outside.

CLASS II - The most utilized class of BSC on campus. Two varieties of Class II BSCs are used and both are adequate for manipulations of RG2 or RG3 pathogens.

- **CLASS II TYPE A**—recirculates 70% of the internal air and exhausts 30% of filtered air into the laboratory. **Volatile chemical or radioactive material should NOT be used in this cabinet.**
- **CLASS II TYPE B**—either recirculates 30% of internal air and exhausts 70% of filtered air through a duct to the outside atmosphere or has 100% total exhaust cabinets. Because of the greater safety margin, small amounts of nonvolatile chemical carcinogens or radioactive materials can be used in this cabinet.
- Since 2002, the National Sanitation Foundation (NSF) has adopted a new classification system. A table comparing the current and pre-2002 BSC classification is shown below:

New NSF BSC Classification	Pre-2002 BSC classification
A1	Class II, Type A
A2	Class II, Type A/B3
A2	Class II, Type B3
B1	Class II, Type B1
B2	Class II, Type B2

CLASS III - cabinets are totally enclosed glove boxes and are used only for the most hazardous biological operations. Class III BSCs have dedicated, independent exhaust fans. These enclosures should not be confused with anaerobic chambers.

Horizontal laminar flow clean benches are not biological safety cabinets and should never be used for work with potentially hazardous materials, whether biological or chemical. These devices protect the material in the cabinet but not the worker or the environment. Similarly, chemical fume hoods are not biological safety cabinets. They draw air in, potentially protecting the worker, but do not protect the material in the cabinet (your sample), and exhaust aerosolized material and vapors/gases into the environment.

Many BSCs have ultraviolet lamps inside them. These lamps provide only limited ability to inactivate microbes. Efficacy is limited to exposed surfaces and penetration of organic material is poor. Note that effectiveness decreases as the lamp ages. Furthermore, exposure to the ultraviolet light may cause eye damage. **Therefore, ultraviolet lamps are not recommended to be the sole source of decontamination of BSC surfaces.**

(b) BSC OPERATION

- **START UP**
  - Turn on blower and fluorescent light.
  - Wait at least two minutes before loading equipment. This is to purge the BSC of contaminated air.
  - Check grilles for obstructions
  - Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
  - Adjust the sash to proper position; NEVER use above the 8-inch mark.
  - RESTRICT traffic in the BSC vicinity. To ensure proper functioning of a BSC, it is best to locate them away from high-traffic areas and doorways to common areas.

- **LOADING MATERIALS AND EQUIPMENT**
  - Load only items needed for the procedure.
  - Do not block the rear or front exhaust grilles.
  - Disinfect the exterior of all containers prior to placing them in the BSC.
  - Arrange materials to minimize movement within the cabinet.
  - Arrange materials within the cabinet from CLEAN to DIRTY (or STERILE to CONTAMINATED).
  - Materials should be placed at least six inches from the front BSC grille.
  - Never place non-sterile items upstream of sterile items.
  - Maintain the BSC sash at proper operating height, approximately level with your armpits.

- **RECOMMENDED WORK TECHNIQUE**
  - Wash hands thoroughly with soap and water before and after any procedure.
  - Wear gloves and lab coat/gown; use aseptic technique.
  - Avoid blocking front and back grilles. Work only on a solid, flat surface; ensure chair is adjusted so armpits are at elevation of lower window edge.
  - Avoid rapid movement during procedures, particularly within the BSC, but in the vicinity of the BSC, as well.
  - Move hands and arms straight into and out of work area; never rotate hand/arm out of work area during procedure.

- Two people working together in one BSC is discouraged, however in the event it is necessary ensure that both workers are following the correct precautions.

- **FINAL PURGING AND WIPE-DOWN**
  - After completing work, run the BSC blower for two minutes before unloading materials from the cabinet.
  - Disinfect the exterior of all containers BEFORE removal from the BSC.
  - Decontaminate interior work surfaces of the BSC with an appropriate disinfectant.

- **DECONTAMINATION AND SPILLS**
  - All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. The final surface decontamination of the cabinet should include a wipe-down of the entire work surface. Investigators should remove their gloves and gowns, and wash their hands as the final step in safe microbiological practices.
  - Small spills within the BSC can be handled immediately by covering the spill with absorbent paper towels, carefully pouring an appropriate disinfectant onto the towel-covered spill, and removing the contaminated absorbent paper towels and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the walls of the cabinet interior, should be immediately wiped with a towel dampened with disinfectant. Gloves should be changed after the work surface is decontaminated. Hands should be washed whenever gloves are changed or removed.
  - Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. The drain pan should be emptied into a collection vessel containing disinfectant. Drain pan should be wiped down with 70% alcohol to prevent corrosion. Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

(c) MAINTENANCE

To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be certified and leak tested. The University of Chicago requires that all BSCs be certified annually by a professional who has been

certified by the National Sanitation Foundation. **This is imperative for BSCs intended for work at BSL-2 or above**.

(d) DRIP PAN MAINTENANCE

Beneath the BSC work surface is a drip pan to collect large spills. This area ought to be routinely checked for cleanliness and, if a major spill has occurred, appropriately cleaned and disinfected (see **DECONTAMINATION AND SPILLS** above).

(e) PURCHASING A BSC

Before ordering a biological safety cabinet, consult the Office of Biological Safety (773-834-2707) for an evaluation of its suitability for the intended research and the available space.

(f) BSC TRAINING

BSC training is offered by the Office of Biological Safety as part of rDNA/BSL-2 training. Contact the OBS to arrange this training.