OAKWOOD UNIVERSITY BIOSAFETY POLICIES AND PROCEDURES MANUAL

GENERAL PRINCIPLES OF BIOLOGICAL SAFETY

Risk Assessment

Risk assessment is the rational application of safety principles to available options for handling hazardous materials. The following characteristics are considered when evaluating a potential pathogen:

- The agent's biological and physical nature
- The concentration and suspension volume of the agent
- The sources likely to harbor the agent
- Host susceptibility
- The procedures that may disseminate the agent
- The best method to effectively inactivate the agent

Risk Groups

Microorganisms that are human pathogens can be categorized into risk groups (RG) based on the transmissibility, invasiveness, virulence (i.e., ability to cause disease), and the lethality of the specific pathogen. Risk groupings of infectious agents (RG1 through RG4) approximately correspond to biosafety levels (BSL1 through BSL4), which describe containment practices, safety equipment, and facility design features recommended for safe handling of these microorganisms. A parallel series of animal biosafety levels (ABSL1 through ABSL4) applies to handling of infected or potentially infected animals.

Beginning with RG1 agents, which are nonpathogenic for healthy human adults, the scheme ascends in order of increasing hazard to RG4. The risk group listing of the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (see website) is an accepted standard, even when recombinant DNA technology is not used. The American Biological Safety Association (ABSA) also provides a comprehensive risk group listing that references agencies globally. The Pathogen Safety Data Sheets (PSDSs)

available through the Public Health Agency of Canada website are an excellent source of information about pathogens.

RISK GROUP 1 agents are not associated with disease in healthy adult humans. Examples: *E. coli* K-12, *Saccharomyces cerevisiae.*

RISK GROUP 2 agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Examples: enteropathogenic *E. coli* strains, *Salmonella*, *Cryptosporidium*, *Staphylococcus aureus.*

RISK GROUP 3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). Examples: human immunodeficiency virus, *Brucella abortus*, *Mycobacterium tuberculosis*.

RISK GROUP 4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). Examples: Ebola virus, Macacine herpesvirus 1 (Herpes B or Monkey B virus).

Consideration of the risk group assignment, however, merely is a starting point for the comprehensive risk assessment. Further attention must be given to the circumstances, such as the planned procedures and the available safety equipment. Then, the recommended precautions may be increased or decreased relative to those based solely on the risk group assignment and adjusted to reflect the specific situation in which the pathogen will be used.

Microorganisms in RG1 require use of standard basic biological laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities and practices. Some of the agents likely to be handled experimentally at Oakwood are *RG2 or RG3 pathogens*; designated as moderate and high hazard, respectively. These agents typically require more sophisticated engineering controls (e.g., facilities and equipment) than are available in standard laboratories, as well as special handling and decontamination procedures.

Consideration also is extended to microorganisms that cause diseases in animals and/or plants, which are not categorized into risk groups as are human pathogens. The desired containment for animal and plant pathogens is based on the severity of the disease, its ability to disseminate and become established in the local environment, and the availability of prophylactic treatment. The relationship between Risk Groups and Biosafety Levels, practices, facilities, and equipment is provided in Table 1.

The progression from invasion to infection to disease following contact with an infectious agent depends upon the dose, route of transmission, invasive characteristics of the agent, virulence and resistance of the exposed host. Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. Attenuated strains should be handled with the same precautions as the virulent

strain unless the reduced pathogenicity is well documented and is irreversible. Viral vectors, even if rendered replication defective, still may pose a threat of recombination with wild-type strains and/or unintentional delivery of their foreign genes. It is prudent to assume virulence and to handle such agents with precautions appropriate for the virulent parental organism.

Relationship of Risk Groups to Biosafety Levels, Practices, Facilities, and Equipment

Risk Group (RG)	Biosafety Level (BSL)	Examples of Laboratories	Laboratory Practices	Facilities and Equipment[a]
RG 1	BSL 1	Basic teaching and research	Good microbiological technique (GMT)	None required; open bench work; directional air flow
RG 2	BSL 2	Primary health services; research; diagnostic, teaching and public health	BSL 1 practices plus protective clothing; biohazard sign	Open bench plus biological safety cabinet (BSC) for potential aerosols; directional air flow
RG 3	BSL 3	Special diagnostic and research	BSL 2 practices plus special clothing, controlled access, directional airflow	BSC and/or other primary containment devices for all activities; directional air flow
RG 4	BSL 4	Dangerous pathogen unit	BSL 3 practices plus airlock entry, shower exit, special waste disposal	Class III BSC or positive pressure suits, double-door autoclave, filtered exhaust air

The risk group assignment should be done along with considering other factors such as the planned procedures as well as safety equipment. The recommended precautions may be increased or decreased based on the risk group assignment and adjusted for the specific situation.

Routes of Infection

Pathogens can be transmitted via several different routes in the laboratory. The most common routes of infection are inhalation of infectious aerosols or dusts, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, animal bites, or percutaneous self-inoculation (injection or incision). Increased risk is associated with pathogens that are aerosol transmitted and when high concentrations or large volumes are used. Appropriate precautions can be implemented to avoid such exposures.

Inhalation of infectious aerosols is implicated as the cause of many laboratory-acquired infections. Even pathogens that normally do not cause infections by inhalation route present a danger when aerosolized. Aerosols can spread throughout the laboratory by traveling along air currents and can contaminate areas considered to be "clean." This creates the potential for indirect laboratory acquired infections to occur. This is a problem for both infectious material and recombinant material. Activities that have the potential to create aerosols should be performed in a biological safety cabinet (BSC) whenever possible (or a fume hood when working with biological toxins). The BSC captures aerosols on a HEPA filter, protecting the worker and the work environment. If the activity cannot be performed in a BSC, additional personal protective equipment (PPE) such as a respirator should be considered.

Some situations warrant special considerations or measures to prevent infection of laboratory personnel by certain microorganisms. For example, organisms which are not known to cause infection in healthy individuals but are known pathogens of persons who have been compromised in various ways including, open wounds, cuts, antibiotic therapy, persons with immune systems rendered deficient via infection, acquired or congenital condition or via therapy (e.g., HIV+, diabetes, complement deficiencies, severe asthma, organ transplant, chemotherapy or long-term steroid treatment) and persons with immunocompromised, immunosuppressed or susceptible immune status (e.g., pregnant women, very young or old, diabetes, individuals on steroid therapy).If any of these conditions apply to you, inform your personal physician/health care professional of your work.

Clinical and Pathological Specimens

Every specimen from humans, cadavers, or animals may contain infectious agents. Human specimens should be considered especially hazardous. Personnel in laboratories and clinical areas handling human blood or body fluids should practice Universal Precautions, an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens. Such personnel are required by OSHA to complete bloodborne pathogen training and be immunized for HBV immunization before attending clinical sessions.

Cultures

Routine manipulations of cultures may also release microorganisms via aerosol formation:

- Popping stoppers from culture vessels
- Opening vessels after vigorous shaking or vortexing
- Flame-sterilizing utensils, which causes spatter
- Electroporation
- Centrifugation
- Sonicating, homogenizing, blending or grinding tissues
- Expelling the final drop from a pipette

Manipulate cultures of infectious material carefully to avoid aerosols. Centrifugation should

involve the use of gasket-sealable tubes and rotors. Seal microplate lids with tape or replace the lids with adhesive-backed Mylar film. Load, remove, and open tubes, plates, and rotors within a BSC or fume hood. Accidental spilling of liquid infectious cultures is an obvious hazard due to the generation of aerosols (airborne droplets containing microorganisms).

Equipment used for manipulations of infectious materials, such as sonicators, flow cytometers, cell sorters, and automated harvesting equipment, must be evaluated to determine the need for secondary containment and to consider decontamination issues. When preparing aliquots of infectious material for long-term storage, consider that viable lyophilized cultures may release high concentrations of dispersed particles if ampoules are not properly sealed. Breakage of ampoules in liquid nitrogen freezers may also present hazards because pathogens may survive and disperse in the liquid phase.
Use of human or animal cell cultures in laboratories requires special consideration. When a cell culture is inoculated with or known to contain a pathogen, it should be classified and handled at the same biosafety level as the agent. BSL2 containment conditions are used for cell lines of human origin, even those that are well established, such as HeLa and Hep-2, and for all human clinical material (e.g., tissues and fluids obtained from surgery or autopsy). Cell lines exposed to or transformed by an oncogenic virus, primate cell cultures derived from lymphoid or tumor tissue, and all nonhuman primate tissues are handled using BSL2 practices. A BSC, not a laminar flow clean bench, should be used for manipulations that have potential to create aerosols. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory.

Animals
Exercise care and thoughtfulness when using animals in research. Numerous risks may be present when animals are used in studies of microorganisms, as well as studies of hazardous chemicals. Use containment and PPE that protects against both the biological and chemical hazards. Precautions commonly include use of a lab coat, gloves and eye protection when handling animals and their bedding; respiratory protection may be recommended when specific conditions present a concern.

There are some inherent risks in working with animals (e.g., allergenicity, bites, and scratches). Laboratory and wild-trapped animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted microorganisms. Even in the absence of known hazards, animal care providers should use precautions to avoid exposure to animal allergens.
In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols.
Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols and dust when changing bedding and cleaning cages. Containment equipment such as a fume hood or biosafety cabinet is sometimes appropriate for performing cage changes. Bedding from animals infected or potentially infected with pathogens must be decontaminated prior to disposal, typically by autoclaving.
Transfer of human cells, primate cells or opportunistic microbes, whether newly isolated or well- established, into immunocompromised animals could result in propagation of pathogens that would be suppressed in the normal host. BSL2 containment must be applied to militate

against such risks and also to prevent spread of animal pathogens within a research colony.

Plant Biocontainment

Biosafety principles are applied to activities involving plants that are exotic, recombinant, and/or grown in association with pathogenic or recombinant microbes and/or pathogenic or recombinant small animals (insects, etc.). Under special circumstances, which typically require explicit approval from USDA-APHIS (U.S. Department of Agriculture-Animal and Plant Health Inspection), it is possible to conduct field trials. Otherwise, release to the environment must be prevented.

The goal is to protect the environment, not the researcher. The risk assessment considers the specific organism(s), geographic/ecological setting, and available mechanical barriers; the selected practices are tailored to the specific situation. It becomes especially difficult to prescribe containment when genetic modifications lead to uncertainty in characteristics such as host range and competitiveness. Containment may be achieved by a combination of physical and biological means. Containment for transgenic plants and their associated plant pathogens relies more heavily on biological factors than is the norm for human and animal infectious agents.

Preventing the spread or release of transgenic pollen is a form of biological containment which can be achieved by using sterile lines, altering day length to prevent flowering, and other strategies.

For research involving plants, four biosafety levels (BSL1-P through BSL4-P) are utilized (see *Appendix P*, NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*).

BSL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and horticultural practices. BSL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BSL2-P and BSL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. Additional facility requirements are also implemented for BSL2-P and BSL3-P containment.

When conducting work in greenhouse space, it is important to communicate the risks and proper precautions required for your project to facility personnel and other researchers sharing the space.

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 and exposure of personnel should an accidental release occur.

Practices and Procedures
The following practices are important not only for preventing laboratory infection and disease, but also for reducing contamination of experimental material.

Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment. Please also see the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) website at http://www.cdc.gov/biosafety/publications/index.htm (Section IV) regarding information on Laboratory Biosafety Level criteria, etc. It is the responsibility of all laboratory staff to effectively decontaminate equipment before it is removed from the laboratory for maintenance, relocation, sale, or disposal.

These standardized practices and procedures provide the foundation for the more restrictive containment of RG3 organisms, which are not covered in this manual. Specialized facilities and rigorous attention to procedures that control the biohazards are required for the conduct of research under BSL3 containment, which must be described in a biosafety manual that is specific to the agents, facilities, and activities. Requirements for the BSL3 manual can be obtained from the website.

Good Microbiological Technique and Personal Hygiene: Biosafety Level 1
- ✓ Do not eat, drink, chew gum, use tobacco, apply cosmetics, or handle contact lenses in the work area.
- ✓ Do not store food for human consumption in the work area.
- ✓ Do not store items such as coats, handbags, dishes or other personal items in the laboratory.
- ✓ Wash hands frequently after handling infectious materials, after removing gloves and protective clothing, and always before leaving the laboratory.
- ✓ Keep hands away from mouth, nose, eyes, face, and hair.
- ✓ Use mechanical pipetting devices; never mouth-pipette.
- ✓ Wear pants (or other clothing that covers legs) and close-toed shoes.
- ✓ Wear appropriate Personal Protective Equipment. A lab coat and eye protection is the minimum required PPE to enter the laboratory, with gloves, respiratory protection, face protection, etc. added as required to suit the activities.
- ✓ Keep laboratory doors closed.
- ✓ Aerosol generating procedures should not be performed in equipment corridors not located with research suites.
- ✓ Plants and animals not associated with the work being performed should not be permitted in the laboratory.

Laboratory Procedures for Handling Infectious Microorganisms: Biosafety Level 2
- ✓ Prepare a site-specific laboratory safety manual outlining activities and defining standard operating procedures.
- ✓ Train employees and ensure that all personnel are informed of hazards.
- ✓ Plan and organize materials/equipment before starting work.
- ✓ Keep laboratory doors closed; limit access to personnel who have a need to be in the laboratory.

- ✓ Post a biohazard sign at the laboratory entrance when RG2 pathogens are used. Identify the agents in use and the appropriate emergency contact personnel. Biohazard signs and laboratory information signs are available from the Office of Biological Safety.
- ✓ A lab coat and eye protection is the minimum required PPE to enter the laboratory. A fully fastened lab coat, gloves, and eye protection must be worn when working with infectious agents or potentially hazardous materials, including human blood, body fluids, tissue and cells.
- ✓ Remove all protective clothing, including gloves, and leave within the laboratory before exiting.
- ✓ When practical, perform all aerosol-producing procedures such as shaking flasks, grinding tissue, sonicating, mixing, and blending in a certified biological safety cabinet. Note that some equipment may compromise cabinet function by disturbing the air curtain.
- ✓ Centrifuge materials containing infectious agents in unbreakable, closable tubes. Use a rotor with a sealed head or safety cups, and load it in a biological safety cabinet. After centrifugation, open the rotor and tubes in a biological safety cabinet.
- ✓ Avoid using hypodermic needles whenever possible. If it is necessary to use them, discard used syringe-needle units in a sharps container without removing or re-capping the needles.
- ✓ Cover counter tops where hazardous materials are used with plastic-backed disposable paper to absorb spills; discard it at the end of the work session.
- ✓ Routinely wipe work surfaces with an appropriate disinfectant after experiments and immediately after spills.
- ✓ Routinely decontaminate all infected materials by appropriate methods before disposal.
- ✓ Report all accidents and spills to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol, the location of cleanup equipment and the *Incident Report Form available through OUHealth Services*
- ✓ Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Establish the habit of weekly cleaning.
- ✓ Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate warning signs.

Laboratory Procedures for Handling Infectious Microorganisms: Biosafety Level 3

Details for BSL3 laboratories may be found in the Biosafety in Microbiological and Biomedical Laboratories (BMBL). Briefly, some points include:

- ✓ Special consideration for all sharps required.
- ✓ Elimination or reduction of the use of glassware in the laboratory.
- ✓ Hazard communication and training for microbes handled in the laboratory.
- ✓ Laboratory BSL3 manual is required.
- ✓ All procedures for infectious materials must be conducted within a BSC.
- ✓ Researchers wear protective clothing with solid-front gowns, scrub suits or coveralls. This is not worn outside of the laboratory.
- ✓ Eye and face protection is worn for anticipated splashes.
- ✓ Gloves must be worn and not be worn outside of the laboratory.
- ✓ Laboratory doors must be self-closing and access restricted.
- ✓ Laboratory must have a ducted ventilation system and laboratory must be able to identify

the direction of the airflow.

- ✓ Facility design including decontamination, engineering controls, operational parameters, SOPs and manuals are specific to each laboratory space. Please contact the respective *Lab Supervisor* for more detailed information.

Biosafety Manual: Required Elements for Laboratories

The Biosafety Manual is required for each of the Laboratories on Oakwood Campus.

Although this list is not inclusive, all elements below must be addressed in the BSL3 manual. Some of the elements may not be applicable to your laboratory and are *italicized* in the list below:

1. Title, date of current revision, table of Contents
2. Emergency Contacts
3. Biosafety Level 3 (BSL3) Description:
    a. Standard Microbiological Practices
    b. Special practices
    c. Safety Equipment
    d. Laboratory Facilities
4. Animal Biosafety Level 3 (ABSL3) Description:
    a. Standard Microbiological Practices
    b. Special practices
    c. Safety Equipment
    d. Laboratory Facilities
5. Microbes

    List agent and describe brief history, host range, route of transmission, biosafety level practices recommended, genetic manipulation performed on the agent (how that affects the risk assessment), other important information to understand the risk of manipulating the agent.
    May also develop a laboratory specific medical response sheet
6. Facility Design and Specifications
    a. How many rooms, location of suite.
    b. Engineering controls (e.g. filters, exhaust, air handling systems, pressure gauges)
    c. Generator for power outages
    d. Security description (e.g. fingerprint scanners, ID card scanners, high security keys)
    e. Waste collection system
7. Facility Certification
    a. Annual certification, work stoppage, certifications performed (BSC, Fume hoods, filters, air handling, showers, HVAC, regular maintenance
8. Facility Decontamination

a. Yearly requirements
b. At the end of facility use
c. When significant repairs are needed

9. Pest Control
    a. Insect and Post Control Program written and in place, reviewed annually.
    b. Describe regulation requirements.
10. Plants, Pets
    a. Describe regulation requirements.
11. Personnel Requirements
    a. List specific requirements for staff
        i. Training
        ii. Any applicable background checks, clearances, evaluations
        iii. Understanding of hazards
        iv. Understanding health and medical requirements
        v. Describe fit testing of respirators (testing, medical clearance, refer to detailed SOP for respirator use)
        vi. Understand and comply with any agent specific programs (quarantine, allergy etc.)
    b. List specific requirements for visitors:
        i. Escort
        ii. Use of PPE
        iii. Visitor training
        iv. Visitor log
        v. Vaccination requirements
    c. Special Maintenance staff requirements as applicable
12. Personnel Training
    a. Describe training procedures.
    b. List SOPs required proficiency.
    c. Specify responsibility of staff.
13. Entrance and Exit Requirements and Personal Protective Equipment (PPE)
    a. List step by step what staff need to do, wear, sign referencing a more detailed SOPs used for training. This list should be specific enough for readers to understand steps but does not have to be as detailed as the SOPs for Exit and Entry.
    b. If separate animal areas are also used, there needs to be a description of exit and entry for those as well and detailed training SOPs.
14. Removal of equipment from the BSL-3 area (e.g. maintenance, repair, replacement)
    a. Detail decontamination procedures and documentation
15. Decontamination of Laboratory Waste
    a. Autoclave use

b. Documentation
c. Efficacy testing
d. Equipment decontamination (large and small) procedures
e. Animal waste decontamination (cages, waste, bedding, animals)
f. Sharps handling

16. Laboratory Research Practices
    a. List specific practices beyond the already required inherent BSL1 and BSl2 practices required to work at BSL3.
    b. Describe any special practices for specific agents (agent A may not be worked on when agent B is being worked on).
    c. Experimental procedures must be well thought out and described in separate SOP's. These may be referenced, but give brief descriptions (e.g. use of BSCs, movement of samples from BSL3 to BSL2, maintenance of vacuum lines).
    d. Cleaning and maintaining equipment and surfaces (e.g. frequency, disinfectant exposure time and concentration, eye wash maintenance).
    e. Animal experimental procedures describe briefly and reference specific SOPs. (cleaning, housing, monitoring, PPE, who performs tasks).
    f. Procedures for waste removal.
17. Special Practices
    a. Consider regulations as applicable to DURC
18. Spill Protocols and Response
    a. Detail Spill protocol with emergency contacts listed and reporting procedures
        i. Inside and outside containment
19. Health and Medical Monitoring
    a. Depending on the agent, certain restrictions, vaccinations or monitoring may need to be in place for BSL3 work.
    b. Outline as applicable to your agent (symptoms for each agent, reporting, what to do when you are sick, emergency procedures, contact numbers, testing requirements)
    c. Types of accidental exposure list:
        i. Needle stick
        ii. Animal Bite
        iii. Break in PPE
        iv. Broken vessel outside BSC
        v. Unknown exposure with symptoms
20. Emergency Response
    a. Accident/Fire/Weather Emergency
    b. Exposure procedures
    c. Breech of containment

    d. Theft/missing agents
21. Shipping and Receiving Requirements
    a. Detail training for shipping and receiving materials
22. Training
23. Policy Documentation
    a. Signature signifying that the BSL3 manual has been read and understood and all questions and concerns have been addressed.
24. Revision history
    a. Specific changes made, who made the changes, date and signature of PI

Personal Protective Equipment

Laboratory coats provide a barrier that protects the worker from hazardous materials contacted in the laboratory. Note that it is not possible to see residues of many hazardous materials; they could have been left behind on various surfaces by another worker. By removing your lab coat when exiting the laboratory, contaminants remain in the laboratory. It follows logically then that protective clothing should not be taken home for cleaning. Depending on the nature of the work, protective clothing also could include disposable sleeves, coats that close in back, disposable protective suits (e.g., Tyvek) and hair and shoe covers.

Gloves should be worn whenever there is the potential for contact with hazardous materials. They further serve to maintain the integrity of the material being handled. Many different types of gloves are available, and the choice depends on the nature of the hazard. Gloves must be removed in a prescribed manner before exiting the laboratory. Material that is transported outside the laboratory that poses a risk to personnel should be surface decontaminated and placed in a clean secondary container so that a lab coat and gloves need not be worn outside the laboratory.

The eyes and mucous membranes are vulnerable routes of exposure. Eye protection should always be worn in the laboratory. Contact lenses may be worn with discretion and in combination with eye protection. Depending on the activities, it may be appropriate to use safety glasses with side shields, goggles, and/or a splash shield.

Respiratory protection should be considered carefully and used only when there is risk of aerosol exposure that cannot be mitigated through the use of alternative procedures or containment equipment. The background level of microbes in the research laboratory should be negligible when good microbiological techniques are employed. Selection of a respirator to guard against pathogens is not as simple as for chemical hazards where tables of permissible exposure limits are available and background levels are factored into the decision. Recommendations for respirators are not documented for work with pathogens with the exception of clinical specimens containing *Mycobacterium tuberculosis* since acceptable exposure levels have not been determined.

An issue regarding respiratory protection is that, if used improperly, the user has a false sense of security. A surgical mask or common dust mask, have poor fit to the contours of the face,

provide minimal protection against large particles and are inappropriate for work with infectious agents.

A HEPA (high efficiency particulate air) filtered face piece (e.g., N95 or N100) is appropriate for many situations where protection against animal allergens and microbes is desired, but the protection will only be as good as the respirator's fit to the face. Furthermore, HEPA filtration is ineffective against volatile chemicals. A full head cover with a Powered Air Purifying Respirator (PAPR) is used when respiratory protection is critical for work with highly pathogenic microbes or in situations where a biological safety cabinet cannot be used. A medical evaluation to wear a respirator, fit testing, and training in proper use are mandatory if respiratory protection is required by the employer.

Engineering Controls
Table 2 describes the relationship between biosafety levels and engineering controls, which include laboratory design, laboratory ventilation, and biological safety cabinets.

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 Research should be contacted for changes or projects that affect laboratory ventilation.

Laboratory Ventilation
For containment in a laboratory to be effective, 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 or adjacent rooms. **To maintain negative room pressure, laboratory doors must be kept closed.** 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, air exhausting from a hazardous facility should be filtered through certified HEPA (high efficiency particulate air) filters that are tested at least annually and verified to retain microorganisms.

Table 2. Summary of Facility Standards Recommended for Biosafety Levels

	BSL 1	**BSL 2**	**BSL 3**
Laboratory visit by Office of Biological Safety	Desirable	Yes	Yes
Isolation of laboratory from public areas	---	---	Desirable
Eyewash, plumbed	Desirable	Yes	Yes
Interior surfaces (impervious, cleanable):	Yes	Yes	Yes
Bench tops	Yes	Yes	Yes
Laboratory furniture	Yes	Yes	Yes
Floors, conventional (no carpet)	Yes	Yes	---
Floors, seamless, integral cove base	---	Desirable	Yes
Ceiling, conventional	Yes	Yes	---
Ceiling, permanent	---	---	Yes

Sinks in laboratory	Yes	Yes	Yes
Hands-free	---	---	Yes
Water supply protected	---	---	Yes
Windows allowed	Yes	Yes	Yes
May be opened	No	No	No
Must be sealed	No	No	Yes
Room penetrations sealed for gas decontamination (pressure decay testing)	No	No	Desirable
Ventilation (single-pass supply/exhaust)	Yes	Yes	Yes
Inward air flow (negative pressure)	Yes	Yes	Yes
Mechanical, centralized system	Yes	Yes	Yes
Mechanical, independent system	No	No	Desirable
Filtered exhaust required	No	No	Desirable
Interlocked supply required	No	No	Yes
Annually test filters/HVAC systems	No	No	Yes
Annually test controls/alarms	No	No	Yes
Doors (self-closing):	Desirable	Desirable	Yes
Double-door entry required	No	No	Yes
Airlock with shower required	No	No	Desirable
Autoclave on site	Desirable	Yes	Yes
In laboratory room	---	---	Desirable
Pass-through (double-ended)	---	---	Desirable
Biological safety cabinets			
Annual certification	Desirable	Yes	Yes
Class I or Class II	---	Desirable	Yes
Class III	---	---	Desirable
Vacuum lines should be protected with liquid trap or in-line HEPA filter	Desirable	Yes	Yes[a]
Waste effluent treatment	---	---	Desirable
Centrifuge with sealed rotors	---	Desirable	Yes

-- not applicable or needed

[a] HEPA filter required

Existing facilities that do not meet these recommendations may need to address deficiencies during future maintenance or remodeling. Contact the Office of Biological Safety for assistance.

Types of Ventilation Equipment

Be sure you know the differences between chemical fume hoods, clean benches, biological safety cabinets, and isolators. These provide three basic types of protection:

- **Personal protection** is the protection of the people working in the laboratory.
- **Product protection** is the protection of the product or experiment.
- **Environmental protection** is the protection of the environment outside the laboratory.

Different types of ventilation equipment provide different types of protection (see Table 3).

Chemical Fume Hoods

Characteristics of chemical fume hoods are that they:

- Offer only protection of personnel.
- Always exhaust air to the outside.
- Do not offer protection to the product or the environment, as there is no filtration of intake and exhaust air; sometimes air cleaning treatment is added to the exhaust.
- Directly draw air from the laboratory over the product in the hood.

Chemical fume hood applications:

- Used for work with chemical hazards; also used to prevent laboratory exposure to biological materials when product protection (sterility) is not a concern.

Clean Benches, Clean Air Devices

Characteristics of clean benches and clean air devices are that they:

- Provide product protection only.
- Create a unidirectional airflow generated through a HEPA filter to provide product protection.
- Discharge air goes across the work surface and directly into workroom.

Clean bench and clean air device applications:

- Any application where the product is not hazardous but must be kept contaminant free.
- Preparation of nonhazardous mixtures and media.
- Particulate-free assembly of sterile equipment and electronic devices.

Biological Safety Cabinets (BSCs)

Characteristics of BSCs are that they:

- Are designed to contain biological hazards and to allow products to be handled in a clean environment.
- Have an inward airflow for personal protection.
- HEPA-filter exhaust air for environmental protection.
- HEPA-filter supply air for product protection (except Class I).

BSCs are separated into classes and types: Class I, Class II (Type A1/A2/B1/B2), Class III (glove box, isolator).

BSC applications:

- Microbiological studies.
- Cell culture research and procedures.
- Protection against hazardous chemicals varies according to the class and type.
- Pharmaceutical research, manufacturing, and quality control testing.

Biological Safety Cabinets

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. When certified and used correctly in conjunction with good microbiological techniques, they can control infectious aerosols. BSCs are designed to provide personal, environmental, and product protection when appropriate practices and procedures are followed. An excellent reference is *Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets,* published by the CDC and NIH. **Clean air devices are not biological safety cabinets and should never be used for work with potentially hazardous biological or chemical materials.** These devices protect the material in the cabinet but not the worker or the environment.

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. Table 3 summarizes the major characteristics of the various types. Four varieties of Class II biological safety cabinets are used on campus. All are adequate for manipulations of pathogens in RG2 or RG3.

Please note that because of the greater safety margin, small amounts of volatile chemical toxins or radioactive materials can be used in Type B cabinets. Type A cabinets, however, recirculate a high percentage of air and therefore cannot be used with toxic, explosive, flammable, or radioactive substances. Class III cabinets and isolators are totally enclosed glove boxes, which are used for the most hazardous biological operations and for super-clean manufacturing. These enclosures should not be confused with anaerobic chambers.

For details about Safety Cabinets please refer to
NSF Standard 49 and Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, Current Edition. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health
Or Contact OU Office of Research

Overview for Proper Use of a BSC

Loading Materials/Equipment and BSC Startup

- ✓ Always close doors to laboratory when working with any biohazardous materials.
- ✓ Turn on blower at least 10 minutes before use and make sure drain valve is closed.
- ✓ Check pressure gauge(s) to ensure proper operating conditions are within range of those indicated on the annual certification label on the BSC.
- ✓ Check grilles for obstructions.
- ✓ Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
- ✓ Disinfect the exterior of all containers prior to placing them in the cabinet.
- ✓ Load only items needed for the procedure.
- ✓ Arrange materials so that movement within the cabinet is minimized; flow of procedure is from clean to dirty. Never place non-sterile items upstream of sterile

items. Check that rear and front grilles are unobstructed. Never hang articles from the interior walls or interior ceiling grid.

- ✓ Once the cabinet is loaded, adjust the view screen to proper position and wait 4 minutes before commencing procedures. Never use the view screen above the mark specified by the certification agency (common opening is 8-inches and up to 12" for animal facilities)
- ✓ Restrict traffic in the vicinity of the BSC.

Recommended Work Technics

- ✓ Wash hands thoroughly with soap before and after procedures.
- ✓ Wear sterile gloves and lab coat/gown and eye protection; use aseptic technique.
- ✓ Avoid blocking front grille. Work only on or over a solid surface and adjust the chair so your armpits are at the level of the lower window edge.
- ✓ Avoid rapid movement during procedures, particularly within the BSC, but also in the vicinity of the BSC.
- ✓ Move hands and arms straight into and out of the work area; never rotate hand/arm out of work area during procedure. Move laterally in work area.
- ✓ Do not use a Bunsen burner that burns gas continuously since the flame causes air turbulence and could cause a fire or explosion. Consider using alternative equipment, such as flameless instrument sterilizers or heat plates.
- ✓ Place contaminated items such as pipettes in a waste receptacle located within the BSC.

Final Purging and Wipe-Down

- ✓ After completing work, run the BSC blower for at least 10 minutes before unloading materials from the cabinet.
- ✓ Disinfect the exterior of all containers before removing them from the work zone.
- ✓ Decontaminate interior work surfaces of the BSC with an appropriate disinfectant effective against the agent used.
- ✓ Routinely check the drip pan beneath the work surface for cleanliness, and if a spill has occurred, clean and disinfect it.
- ✓ Take care to prevent towelettes from being sucked into exhaust plenums.
- ✓ When closing the sash, the BSC blower needs to be turned off unless the BSC is labeled 'Energy efficient engineered'.

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 work zone. Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.

Small spills within the cabinet can be handled immediately by placing the contaminated absorbent paper toweling into the biohazard waste container. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened

with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before clean absorbent toweling is placed in the cabinet. 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. Beneath the BSC work surface is a drip pan to collect large spills. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface, grilles, and 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. If the drain pan is accessible, wipe it down to remove remaining debris. Should the spilled liquid contain radioactive material, Radiation Safety personnel should be contacted for specific instructions on conducting a similar procedure.

Maintenance

To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be certified**.** All biological safety cabinets should be certified annually.

All BSCs must be either surface or gas decontaminated prior to being moved from one space to another. Before a unit is removed from the lab for maintenance, opened up for maintenance or repair, relocated, or disposed, laboratory staff are responsible for arranging surface or gas decontamination.

Disposal of Wastes from Biological Laboratories

Details regarding disposal of biological wastes, liquid wastes, wastes from animal experiments and noninfectious wastes need to be added in here from the respective departments. Decisions are left to the personnel directly involved, provided they are well informed and prepared to verify the effectiveness of the treatment. The choice depends largely on the treatment equipment available, the target organism, and the presence of interfering substances (e.g., high organic content) that may protect the organism from decontamination. Other common factors that influence the efficacy of disinfection are contact time, temperature, water hardness, and relative humidity.

As a general rule, materials that can cut, but are not intended to do so, should be disposed in a manner that prevents harm. Examples of such materials include fragile glass, glass slides and cover slips, and pipettes and pipette tips. If a bag is apt to be punctured because of sharp-edged contents, double bagging and boxing may be necessary. Furthermore, the material must be decontaminated prior to disposal if it harbors infectious agents or recombinant materials.

Methods of Decontamination

Choosing the right method to eliminate or inactivate a biohazard is not always simple; it is difficult to prescribe methods that meet every contingency. Decisions are best left to the personnel directly involved, provided they are well informed and prepared to verify the

effectiveness of the treatment. The choice depends largely on the treatment equipment available, the target organism, and the presence of interfering substances (e.g., high organic content) that may protect the organism from decontamination. Other common factors that influence the efficacy of disinfection are contact time, temperature, water hardness, and relative humidity.

EMERGENCY PLANS

Emergency plans should be tailored for the laboratory. The laboratory supervisor should prepare instructions specifying immediate steps to be taken and all personnel should understand basic emergency measures. It is recommended the instructions be displayed prominently in the laboratory and annually reviewed with personnel. No single plan will apply to all situations, but the following general principles should be considered:

- Always know the location of emergency response materials, such as spill kits, fire extinguishers, eyewashes, safety showers, first aid kits, automated exterior defibrillators (AED), contact numbers or first aid kit.
- In the event of a spill outside of containment, everyone should leave the affected area immediately. Even for apparently small spills, evacuation is important if aerosols were generated. Clothing, if contaminated, should be removed. Exposed skin should be washed for at least 15 minutes with soap and water. A splash to the eyes should be treated by flushing with water at a plumbed eyewash for at least 15 minutes.
- If a spill presents immediate danger to people and exceeds the ability of local staff to control it, the event should be reported as an emergency to OU Police Department (OUPD).
- Close the laboratory door and post a "No Entry" sign indicating the hazard. Notify the laboratory supervisor, OUPD, and the Office of Research.
- Seek medical treatment for persons exposed.
- Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation should be given appropriate first aid and then seek immediate medical assessment.
- If necessary, call OU Police Safety (OUPD) for emergency at any hour.
- Complete a Incident Report Form online at the OUHS website within 24 hours.
- Do not reenter the room until aerosols have settled (30 minutes minimum), and the extent of the hazard and its dissemination has been determined.
- Each person who enters the laboratory for cleanup should wear at a minimum a lab coat, gloves and eye protection.
- Use an appropriately concentrated disinfectant to decontaminate the area. A supply of stock disinfectants should always be available.
- Decontaminate all materials used in cleanup procedures.

In any emergency situation, attention to immediate personal danger overrides containment considerations. With the exception of BSL3 laboratories, properly garbed and masked fire or security personnel are adequately prepared to enter any biological laboratory in an emergency. Reporting is an additional required step in emergency management. The supervisor should always be notified and a Incident Report Form prepared even in situations that do not involve emergency responders or require immediate medical care. Notify the OUPD / Office of Research of any spills outside of containment, potential exposures, or any research-related accidents and illnesses.

Biohazardous Spills
Laboratories should be prepared to immediately address biohazardous spills by training personnel in advance and having appropriate spill-control materials in place. Note that biohazardous materials being transported outside of laboratories, including to autoclaves, should be in secondary containment capable of completely containing the spills.

In addition to spill-prevention procedures, information regarding spill-control procedures should be displayed in laboratories and periodically reviewed with personnel. In the event of emergency, do not hesitate to call 911 if necessary.
All spills or releases of biohazardous or recombinant materials must be reported to the Principal Investigator (PI) and also to the Office of Research and Oakwood University Health Services (UHS) within 24 hours through the use of the *Incident Report Form.*

Recommended Supplies
Appropriate materials to handle biohazardous spills should be prepared in advance, placed in strategic locations inside or outside the laboratory, and all laboratory personnel informed of the location(s).

Disinfectant(s):
- Disinfectant(s) appropriate to the agent(s) used in the lab should be available.
- If dilutions are made in advance (i.e. 10% bleach), fresh solutions should be made (and dated) on a specific schedule depending on the materials and manufacturer's instructions.

Absorbent materials:
- Sufficient absorbent materials should be available to absorb the maximum volume of biohazardous materials handled in the laboratory.
- Paper towels or other absorbent laboratory wipes are commonly utilized, and spill- specific materials are available through laboratory supply companies.

PPE:
- PPE may vary depending on the biosafety level, route of infection of agents, etc., but minimally should include disposable gloves, eye protection, and laboratory coats or gowns.

Other:
- Signage to post the area as off-limits until potential aerosols have settled.

Decontamination Procedures
General spill cleanup procedures are provided below, and may be modified to meet the specific needs of your laboratory.

Spills Inside a BSC
- Properly functioning BSCs should contain potentially dangerous aerosols from spills within the units.
- Immediate Response:
    - Immediately stop all work, but leave BSC blower on during clean-up.

    - Notify others in the area.
    - Determine if first aid and/or medical attention is needed (injury, direct or potential exposure). Call OUPD and 911 if necessary.
    - Remove potentially contaminated PPE and dispose of them in biohazard waste containers inside the BSC.
    - Wash hands thoroughly with soap/antimicrobial agent and water.
- Clean-up Response:
    - Don new PPE (at minimum, gloves, lab coat, eye protection)
    - Completely cover spill with absorbent material and pour an appropriate disinfectant solution onto absorbent material.
    - Flood drain pan (Type II BSC) with disinfectant.
    - Using paper towels and disinfectant, wipe down walls, work surfaces, and equipment.
    - Let disinfectant stand for an adequate length of time (up to several hours).

- Proper disinfection time is dependent on specific disinfectants, organic load, and type of microbe. Consult your disinfectant documentation for further guidance.
    - Flush drain pan with water and remove drain tube.
    - Transfer all contaminated disposable materials into an autoclave bag.
    - Wipe down exterior of autoclave bag, disinfectant container, and other contact surfaces with disinfectant.
- Wrap-up:
    - Remove PPE and dispose of them in biohazard waste containers inside the BSC (autoclave prior to disposal).
    - Wash hands thoroughly with soap/antimicrobial agent and water.
    - Autoclave all contaminated materials.
    - Report incident to PI (if not already notified).

Spills Outside of a BSC

- Immediate Response:
    - Immediately stop all work and notify others in the area.
    - For material infectious via aerosols:
        - Evacuate everyone from the laboratory area
        - Remove potentially contaminated PPE (and potentially contaminated clothing) and dispose of them in biohazard waste containers.
        - Determine if first aid and/or medical attention is needed (injury, direct or potential exposure). Call OUPD / 911 if necessary.
        - In a secondary location, wash hands thoroughly with soap/antimicrobial agent and water.
        - Post all laboratory doors with “Spill: Do not enter” signage.
        - Wait at least 30 minutes before re-entry to allow potentially dangerous aerosols to dissipate.
    - For materials not infectious via aerosols:
        - Determine if first aid and/or medical attention is needed (injury, direct or potential exposure). Call OUPD / 911 if necessary.
        - Remove potentially contaminated disposable PPE and dispose of

them in biohazard waste containers.
    - Wash hands thoroughly with soap/antimicrobial agent and water.

- **Clean-up Response:**
    - Put on new/clean PPE (at minimum, gloves, lab coat, eye protection)
    - Completely cover spill area and area in immediate proximity to the spill with absorbent material and pour an appropriate disinfectant solution onto absorbent material. Pour disinfectant in a controlled fashion in order to minimize aerosols.
    - With disinfectant and absorbent material. Wipe down any equipment or furniture in the spill area that may have been splashed with material.
    - Transfer all contaminated disposable materials into an autoclave bag.

- **Wrap-up:**
    - Remove and discard disposable PPE (autoclave prior to disposal).
    - Wash hands thoroughly with soap/antimicrobial agent and water.
    - Autoclave all contaminated materials.
    - Report incident to PI (if not already notified)
    - Report incident to OU Public Safety

LABORATORY SECURITY AND PUBLIC AREAS

Security commonly refers to safeguarding electronic equipment and personal belongings. Security also needs to be considered in terms of preventing theft of materials from our facilities that have the potential to harm our community.

The degree to which laboratory security is implemented should be commensurate with risk. All laboratories, including those handling only low-risk biological materials under BSL1 containment practices, must maintain a basic level of security. You should make an effort to know all the people who work in your area, and to greet unknown persons who enter laboratories and to ask their purpose. According to CDC's guidance for BSL1 laboratories, "Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens is in progress." Translated into common practice, this statement means that everyone entering a laboratory should have the supervisor's explicit approval to be there.

Security concerns also extend to all laboratory materials in storage. Unauthorized persons should not be able to access it. Inventory records are instrumental to determining if there is a discrepancy due to misuse or a security lapse. An easy way to prevent unauthorized access is to lock the laboratory door when the room is unoccupied. Equipment should be located in the laboratory to prevent theft and release of materials. For materials stored outside of the laboratory, such as in a freezer located in a hallway or shared equipment space, the equipment must be locked at all times.

MINORS

Minors can be granted access to a biological laboratory only for educational activities unless the activity is deemed to have high hazard potential.

This document is subject to review and amendments as required.

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This manual has been adapted from UWMadison Researchers' Biosafety Manual with permission. We thank the Office of Biosafety at UWMadison for giving us the permission for preparing Biosafety Policies and Procedures at Oakwood University.

USEFUL REFERENCES

Note: URLs of remote sites change frequently. Hence, you may need to search from the root directory of each organization.

American Biological Safety Association (ABSA) list of risk groups: http://www.absa.org/riskgroups/index.html

Arthropod Containment Guidelines. Version 3.1 (12/01), A project of the American Committee of Medical entomology of the American Society of Tropical Medicine and Hygiene: http://www.astmh.org/subgroup/archive/ACGv31.pdf

Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH. Current Edition: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.ht

Public Health Agency of Canada, Material Safety Data Sheets (MSDS) for Infectious Substances: http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php

National Sanitation Foundation Standard (NSF) 49, Biological Safety Cabinets, 2002: http://www.nsf.org/business/biosafety_cabinetry/index.asp

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules: https://osp.od.nih.gov/biotechnology/nih-guidelines/

NTP Report on Carcinogens. National Toxicology Program, Department of Health and Human Services: http://ntp.niehs.nih.gov/?objectid=03C9B512-ACF8-C1F3-ADBA53CAE848F635

OSHA Lab Standard. Occupational exposure to hazardous chemicals in laboratories. 29 CFR 1910.1450 Appendix A – National Research Council Recommendations Concerning Chemical Hygiene in Laboratories (Non-Mandatory): http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10106

Public Health Service, U.S. Department of Health and Human Services, CDC/NIH. Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets, Current Edition:

http://www.cdc.gov/biosafety/publications/

TOXNET, a cluster of databases on toxicology, hazardous chemicals, and related areas. The National Library of Medicine: http://toxnet.nlm.nih.gov/

Traynor et al. 2001. A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes. Information Systems for Biotechnology: http://www.isb.vt.edu/Containment-guide.aspx

World Health Organization (WHO) Laboratory Biosafety Manual. 3rd Ed. revised. Geneva, 2004: http://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/