## Biosafety Manual

## April 2015

Standard operating procedures to ensure biological safety

Biological agents (such as bacterial cultures, cultured cells, virus stocks) should be purchased (or otherwise received) in accordance with the biology department’s Biosafety regulations presented in this document.

Biological agents deemed to be hazardous, purchased (or otherwise received), shall be stored in accordance with the biology department’s Biosafety regulations presented in this document.

**Control Measures and Safety Equipment**

Laboratory ventilation should

1. provide a source of air for breathing and for input to local ventilation devices;
2. not be relied on for protection from toxic substances released into the laboratory

Biological Safety Cabinets

Biological safety cabinets referred to in this section are classified as Class I and Class II cabinets. Additional information on biological safety cabinets is published as a CDC-NIH web page: <http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>

Class I - a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater.

Class II - a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan (www.nsf.org).

Prior to being used with microbial pathogens or for animal cell culture / virus work, these cabinets shall be tested and certified by a representative of a company that specializes in biosafety cabinet certification and decontamination. Thereafter, the cabinet shall be recertified annually. A biosafety cabinet certifier may be found by searching the website http://info.nsf.org/Certified/Biosafety-Certifier/.

Biological Agents

Resources for the information in this section include an on-line document posted by the University of Colorado, Boulder Institutional Biosafety Committee as “IBC Requirements for Laboratory Operating Practices, Physical Containment, and Training for Research Involving Biological Agents.” (Document footer: EHS/IBC doc. 2/16/07 dd) (<http://ehs.colorado.edu/BioSafetyDocs/IBCGuidContanLabs.pdf>), Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition (http://www.cdc.gov/biosafety/publications/bmbl5/), Establishing Appropriate Biosafety Laboratory Environments (p. 542-546) in Biological Safety: Principles and Practices. 2006. Fleming and Hunt (editors) 4th edition. ASM Press Washington DC and Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory: Development and Rational (May 2013) Emmert & ASM Task Committee on Laboratory Biosafety in Journal of Microbiology & Biology Education. Changes were made as needed to apply to facilities in the biology department to which this safety document applies. Most importantly, these ‘standards’ parallel the current NIH guidelines.

The James Madison University Institutional Biosafety Committee (IBC), the Biology department’s Chemical Hygiene and Safety officer, and the University Environmental Health Coordinator, require that the following standard and special microbiological practices, physical containment or laboratory design, containment equipment, and training be implemented when using organisms containing recombinant DNA or biological agents that are known or potential biohazards. These requirements include hygienic and operational practices that are critical in providing for a safe work environment and assuring a viable research product is produced. These practices are also necessary for minimizing and/or eliminating the risk of occupational exposure to infectious and potentially infectious substances.

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

Standard Practices and Training

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

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

Physical Containment Levels

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

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. There are four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. The National Cancer Institute describes three levels for research on oncogenic viruses that roughly correspond to NIH BL2, BL3, and BL4 levels. Currently, the facilities within the biology department contain laboratories that require only BL1 and BL2 practices and physical containment. Hence, only ‘standards’ that are appropriate to these biosafety levels are detailed in this document.

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

Biosafety Level 1 (BL1)

BL1 Standard Microbiological Practices

* Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

* Work surfaces are decontaminated at least once a day and after work with infectious materials is finished, and after any spill of viable material is cleaned with disinfectants that are effective against the agents of concern.

* All contaminated liquid or solid wastes are decontaminated before disposal as stipulated later in this section (below)
* Mechanical pipetting devices are used; mouth pipetting is prohibited.

* Policies for the safe handling of sharps are instituted. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant ‘sharps’ container and removed as stipulated later in this section (below).
* Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. No preparation, storage or consumption of food or drink is permitted in the laboratory.

* Persons wash their hands: (i) after handling materials involving microorganisms and animals (ii) before exiting the laboratory

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

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

* A biohazard sign must be posted at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and the phone number of the investigator.

BL1 Special Practices

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

* An insect and rodent control program is in effect.

BL1 Containment Equipment

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

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

* Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated. Protective eyewear should not be shared and should remain in the laboratory. If reused or shared, eyewear must be sanitized appropriately.

BL1 Laboratory Facilities

* Laboratories should have doors for access control.

* The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

* Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

* Laboratory furniture is sturdy. All furniture in the laboratory (chairs, stools, etc.) must be nonporous or non-pervious so that it can be cleaned and disinfected in case of a spill. Spaces between benches, cabinets, and equipment are accessible for cleaning.

* Each laboratory contains a sink for hand washing. Foot, knee, or automatically operated sinks are recommended.

* If the laboratory has windows that open, they are fitted with fly screens.

Biosafety Level 2 (BL2)

BL2 Standard Microbiological Practices

* All procedures for BL1 Standard Microbiological Practices, AND

BL2 Special Practices

 All BL1 Special Practices, AND

* The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections.

* The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

* When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent and the biosafety level, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering and exiting the laboratory (e.g., immunization, personal protective equipment). Please see last page of this document for an example of an appropriate hazard warning sign.
* Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., office, dining hall, library), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

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

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

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

* Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Broken glassware should be promptly placed in a puncture-resistant container and decontaminated if needed.
* Spills and accidents that result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

* Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory.

* 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.

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

BL2 Containment Equipment

* All BL1 Containment Equipment, AND

* Properly maintained biological safety cabinets (Class I or II), preferably Class II, or other appropriate personal protective or physical containment devices are used whenever:

* 1. Procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures
	2. High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

* A properly maintained biological safety cabinet (Class I or II), will have a current, annual certification that under normal operating circumstances the unit performs to manufacturer’s specification.
* Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the biological safety cabinet.

BL2 Laboratory Facilities

* All BL1 Laboratory Facility Requirements, AND

* Provide lockable doors for facilities that house restricted agents.

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

* An eyewash station is readily available.

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

* An autoclave for decontaminating laboratory wastes is available.

**Biological Waste Disposal**

Potentially hazardous biological/physical waste will be removed (from appropriately labeled containers located in biology department laboratories and appropriately packaged for disposal by JMU biology personnel specially trained in handling of these wastes.

**Autoclave Validation**

Autoclaves are used to sterilize and decontaminate biological waste. The key components are:

A. Appropriate use of the autoclave to decontaminate biological waste

 Minimal parameters are 121ͦ C at 15 psi for 15 minutes.

 Time may need to be increased for larger loads and larger volumes of liquid.

 Items should be loaded in manner that ensures that steam can penetrate packages and test tubes.

B. Recordkeeping – There should be a log or notebook adjacent to the autoclave to indicate:

 Date

 Time

 Use name

 Cycle type used (liquid, gravity, and vacuum)

 Items autoclaved (media, waste, etc.)

C. Performance verification

 1. The autoclave tape should be checked prior to opening the door to ensure all temperature, pressure, and/or time parameters were met.

 2. Autoclave indicator tape should be clearly visible on each item placed in the autoclave.

 3. The autoclave operation should be verified monthly using a biological thermophile spore former, *Bacillus stearothermophilus*.

D. Annual calibration and maintenance

 An outside service vendor will perform this service.

BIOSAFETY IN TEACHING LABORATORIES

The CDC and the National Institute of Health (NIH) have developed standard procedures for working with and providing protection against biological hazards. The publication *Biosafety in Microbiological and Biomedical Laboratories* (CDC/NIH, 2009) (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm) provides specific descriptions of combinations of microbiological practices, laboratory facilities, and safety equipment associated with four distinct levels of biosafety required for specific categories of infectious agents. Each biosafety level (BSL) is based on the accepted potential hazard of the agent, as well as the general operations of the laboratory. Generally, BSL-1 is for work with agents that pose minimal hazard, while BSL-4 applies to conditions related to protection against agents posing the greatest hazard. Only BSL-1 and BSL-2 are described here. It is important to note that the choice of BSL for a particular organism or laboratory operation is subject to variance based on specific experiments, procedures, culture volumes, or use of mutagenized pathogens involved. Generally, introductory microbiology teaching laboratories are expected to operate under a minimum of BSL-1 standards, while intermediate to advanced instructional or research microbiology laboratories are expected to function under a minimum of BSL-2 guidelines.

BSL-1

BSL-1 is appropriate for instruction or experimentation involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to the environment. Examples of BSL-1 agents include *Bacillus subtilis, Lactobacillus* species, *Erwinia* species, *Micrococcus luteus, Staphylococcus albus,* and infectious canine hepatitis virus. Students must wear protective laboratory coats (and gloves when appropriate) and wash their hands with soap after they handle viable materials and/or animals, after removing gloves, and before leaving the laboratory. Eating, drinking, chewing gum, handling contact lenses, handling personal electronic devises, and applying cosmetics are not permitted in laboratory work areas. Students at increased risk of infection or who wear contact lenses in laboratories may be instructed to utilize safety glasses, inhalation masks, and/or a face shield as appropriate for the activity at hand. Additionally, mouth pipetting is forbidden; mechanical pipetting devices are required, all procedures are performed carefully to minimize the generation of aerosols, and work surfaces including the instructor’s bench are decontaminated at least once a day and after any spill of viable material. If appropriate, students should receive instruction on the safe handling of sharps.

BSL-2

BSL-2 facilities and precautions are required for instruction or experimentation involving agents of moderate potential hazard to personnel and the environment. Examples of BSL-2 agents include *Staphylococcus aureus,* most *Enterobacteriaceae, Pseudomonas* aeruginosa, *Clostridium* species, *Mycobacterium leprae, Bordetella pertussis, Candida albicans, Cryptococcus neoformans,* and human blood pathogens such as hepatitis B virus (HBV), HCV, and human immunodeficiency virus (HIV). Many of these organisms are frequently encountered in microbiology laboratory courses. Instructors conducting courses that require BSL-2 must have specific training in handling pathogenic agents and must be directed by supervisors or scientists with advanced experience in this regard. Additional precautions against exposure to BSL-2 agents include (i) limiting laboratory access to specific individuals and to periods of time when instruction or experimentation is ongoing under supervision, (ii) using enhanced precautions against injuries due to contaminated sharps and needle sticks, (iii) substitution of plasticware for glassware whenever feasible, and (iv) conducting procedures representing a high risk of generating aerosols only within approved biological safety cabinets or the equivalent. Also, eye-wash stations should be readily accessible in the BSL-2 laboratory area.

Students must wear protective laboratory coats while working in the laboratory, and these are to remain in the laboratory (i.e., not worn into nonlaboratory areas such as cafeterias, libraries, or administrative offices). In addition, gloves are worn when handling infected animals or whenever hands may contact infectious materials, contaminated surfaces, or equipment. Double gloving (wearing two pairs of gloves) is appropriate when handling needles or other sharps. Gloves are not reused or worn beyond the laboratory area, and hands are washed whenever gloves are removed. Proper glove removal video is available at [www.youtube.com/watch?v=dyLEd9cng5U](http://www.youtube.com/watch?v=dyLEd9cng5U). Laboratory coats and other protective clothing should be appropriately decontaminated by the institution whenever contaminated, whenever used when working with highly pathogenic organisms, or whenever they are to be taken out of the laboratory, such as at the end of a school term.

Individuals at increased risk of infection or for whom infection may be unusually hazardous (e.g., immunocompromised or immunosuppressed) are generally not permitted in the laboratory. To whatever extent practical, the laboratory instructor assesses each situation and advises who may enter or work in the laboratory, and establishes policies and procedures whereby only person who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory. In the BSL-2 laboratory, when potential pathogens under use necessitate special considerations for entry (e.g., immunization), a hazard warning sign incorporating the universal biohazard symbol should be posted on access doors to the laboratory. The biohazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirement(s) for entering the laboratory. Further measures may be required for particular cases (e.g., obtaining baseline serum samples and ensuring proper immunization for laboratory personnel and providing specialized training and continuing education on the potential hazards associated with the work involved).

Waste management is similar to that of BSL-1 laboratory. Exposures, spills, or accidents which result in the obvious potential for contamination by infectious agents or toxins must be communicated immediately to the laboratory instructor for appropriate cleanup and medical evaluation. Records are maintained as per institutional guidelines. All cultures and other hazardous waste are either appropriately decontaminated prior to removal from the laboratory or packaged in clearly labeled leak proof containers for contamination elsewhere.

Risk Assessment and Biosafety Levels

The hazards of working with microorganism must be assessed for appropriate safe handling, containment, and disposal. A risk assessment for each laboratory activity and organism is necessary in order to identify the proper procedures and safety equipment needed. Risk assessment determines the biosafety level of the laboratory. A thorough risk assessment takes into account the microorganism(s) being used, the manipulations performed with the organism(s), and the risks inherent in performing the laboratory activity. The microorganism alone does not determine the biosafety level of the laboratory. Manipulations that generate aerosols, create splash potential, or require large volumes of culture increase the risk associated with a particular microorganism. Safety requirement information for specific microorganism may be found at the CDC website ([www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf](http://www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf) ) or Public Health Agency Canada ([www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php](http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)).

Students at Special Risk in Microbiology Teaching Laboratory

Some students may represent special risk considerations in the microbiology laboratory depending on the nature of the organisms handled. Examples of these individuals include:

Immunocompromised or immunosuppressed - those with immune systems rendered deficient through infection (e.g., HIV) or congenital or acquired conditions (e.g., diabetes, complement deficiencies, severe asthma) and/or therapy (e.g., transplant or cancer chemotherapy or long-term steroid treatment). Also students who live with or care for an immune-compromised individual.

Unvaccinated - those whose personal or religious beliefs or country of origin preclude vaccination against pathogens common in teaching laboratories, such as *B. pertussis*, *Corynebacterium diphtheriae*, and *Haemophilus influenzae*, and *Clostridium tetani*.

Trauma - those students with significant risk of direct contact with pathogens through lesions from surgical procedures, burns, or other injuries or predispositions, such as eczema.

Pregnancy - women who are at an increased risk for some type of infections for themselves and the fetus.

Disabled - students with physical disabilities such as neuromuscular illness (e.g., multiple sclerosis), uncorrected vision impairment (e.g., significant loss of sight or color blindness), or substantial hearing loss.

Training Requirements

In addition to the biology safety training form, (Standard Laboratory Practices and Safety Rules, Attachment 3), which is completed during safety training session held at the beginning of each semester, each laboratory instructor shall provide instruction on the health risks involved, especially in a BSL2 course / laboratory. The students will be required to review and sign the BIOSAFETY INSTRUCTION AND VERIFICATION form Attachment 4. Students will also receive appropriate training, especially for BSL2 laboratory courses.