

INSTITUTIONAL BIOSAFETY MANUAL

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BIOLOGICAL SAFETY MANUAL WRIGHT STATE UNIVERSITY

FOREWORD

In October 1994, Wright State University reconstituted the Institutional Biosafety Committee and appointed an Institutional Biological Safety Officer. The charge of the committee was to provide oversight on the use of recombinant DNA (rDNA), infectious biologicals and biological toxins.

One of the first needs identified by the committee was an institutional biosafety manual. The purpose of the manual is not to replace existing biosafety publications as published by the National Institute of Health (NIH) or the Center for Disease Control (CDC), but to provide general information relative to the biological safety program that will assist WSU researchers in:

1. Submitting of protocols consistent for review and approval by the Institutional Biosafety Committee
2. Establishing acceptable practices/procedures in the use of potentially infectious microorganisms and other biohazards
3. Developing a laboratory safety manual for a specific research activity, when necessary
4. Obtaining assistance from the Biological Safety Officer and the Department of Environmental Health and Safety

The Institutional Biosafety Committee has adopted several guidelines and policies relative to the use of rDNA, infectious biologicals and biotoxins. In view of the anticipated frequent addition to and revision of the guidelines and policies, it was determined that it was not practical to place these documents in the Institutional Biosafety Manual. They are, however, accessible directly through the Research and Sponsored Program's (RSP) home page. RSP's home page can be accessed directly through <<http://www.cs.wright.edu/rsp>>.

Appendix F contains a listing of resources available through the World Wide Web and other sources. Examples would include Material Safety Data Sheets (MSDS) for infectious agents, MSDSs for chemicals, available training videos, reference texts, regulations for the Interstate Shipment of Etiologic Agents (49CFR72) and other guides.

The references shown in Section 15 of the manual are available to the researcher and their routine use is encouraged. **Reference No. 5, "CHEMICAL HYGIENE PLAN," in the section titled, "Biological Safety" contains very useful information and can be an excellent reference as a copy of the plan is available in every laboratory. It is important to remember that the researcher has the ultimate responsibility for the safe usage and disposal of potentially infectious materials.**

Class 4 Microorganisms or Biosafety Level 4 requirements will not be addressed in this manual as the university does not now, nor is it anticipated that it will ever, have facilities adequate to handle Class 4 Biohazards.

**INSTITUTIONAL BIOSAFETY MANUAL
WRIGHT STATE UNIVERSITY**

SECTION 1 - Glossary of Abbreviations and Terms

Abbreviations:

1. ABSL - Animal Biological Safety Level
2. BSL - Biological Safety Level
3. CDC - Center for Disease Control
4. EHS - Environmental Health and Safety
5. ESPM - Equipment Surplus Property Management
6. HEPA - High-Efficiency Particulate Air
7. IBC - Institutional Biosafety Committee
8. IBSO - Institutional Biosafety Officer
9. LAR - Laboratory Animal Resources
10. MSDS - Material Safety Data Sheet
11. NIH - National Institutes of Health
12. NSF - National Sanitation Foundation
13. NRC - National Research Council
14. ORDA - Office of Recombinant DNA Activities (NIH)
15. OSHA - Occupational Safety and Health Administration
16. PI - Principal Investigator
17. RAC - Recombinant DNA Advisory Committee (NIH)
18. rDNA - Recombinant DNA
19. RSP - Office of Research and Sponsored Programs

Terms:

Animal Husbandry	A branch of agriculture concerned with the production and care of domestic animals
Biological Toxin	A colloidal proteinaceous poisonous substance that is a specific product of the metabolic activities of a living organism and is usually very unstable, notably toxic when introduced into the tissue, and typically capable of inducing antibody formation
Biosafety Level 3 Facility	A facility specifically designed for the use of Class 3 organisms. Formerly referred to as a P3 facility
Blood-Borne Pathogens	Pathogenic microorganisms that are present in human blood that can cause disease in humans. These pathogens include, but are not limited to HBV and HIV
Baseline Serum	A blood sample drawn from a human for archiving for future reference by a physician
Class I Biosafety Cabinet	An enclosure with an inward airflow through the front opening. Provides protection for the worker and the laboratory environment but not to product being utilized in the cabinet
Class II Biosafety Cabinet	An enclosure with an inward airflow through the front opening. Provides protection to the worker, the environment, and the product being utilized in the cabinet
Class 1 Organisms	Organisms not known to cause disease in healthy adults
Class 2 Organisms	Organisms associated with human disease, infectious through autoinoculation, ingestion, mucous membrane exposure
Class 3 Organisms	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences
Containment	Used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled and maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.
Host	Organism in which the rDNA replicates
Negative Airflow	Directional airflow from areas exterior to a laboratory into the laboratory
Primary (p) Containment	methods to protect the internal laboratory environment
rDNA	DNA prepared by breaking up and splicing together DNA from several different species of organisms
rDNA Insert	That (those) strand(s) of foreign DNA being inserted into the host/vector
Secondary (s) Containment	methods to protect the environment external to the laboratory
Sharps	Any object that can penetrate the skin, e.g., needle, scalpel, knife, etc.
Vector	Carrier used to introduce rDNA into the host system and that facilitates replication

SECTION 2 - Responsibilities

Wright State University is responsible for providing a safe working environment for all University activities and for compliance with all applicable federal, state, and local regulations concerning the use of biological agents, biological toxins, and recombinant DNA. Institutional responsibilities include the establishment and support of an Institutional Biosafety Committee, the appointment of an Institutional Biosafety Officer, and the establishment and support of a Department of Environmental Health and Safety.

A. Chairperson, Institutional Biosafety Committee

1. Ensure that the Institutional Biosafety Committee is properly constituted and fulfills its requirements under the appropriate regulations, rules, etc.
2. Ensure that all members of the Institutional Biosafety Committee are adequately trained in appropriate containment practices, secondary containment procedures, and accidental spill containment procedures to fulfill their responsibilities as members of the Institutional Biosafety Committee.

B. Institutional Biosafety Committee (IBC)

1. Advise the President, Provost, Associate Provosts, Deans, and Department Chairs on matters related to biohazards and biosafety with their respective areas of responsibility.
2. Develop, recommend, and implement policies and procedures for biological risk assessment and biological risk reduction throughout the University.
3. Develop emergency plans for the containment and resolution of accidental spills and other related emergencies with an emphasis on risk reduction, personnel protection, and environmental protection.
4. Oversee all research and teaching activities involving biohazardous agents including review and approval prior to initiation, annual reviews and updates, reviews of laboratory safety equipment and procedures, and certification of compliance with all applicable rules and regulations governing the use of biohazardous materials.
5. As an agent of the Institution, ensure that all principal investigators are sufficiently trained in appropriate containment practices, secondary containment procedures, accidental spill containment, and their responsibilities as principal investigators.
6. Advise and provide technical expertise, whenever possible, to the Institutional Biosafety Officer on matters regarding biosafety
7. Conduct investigation of serious violations or problems and to make recommendations to the Associate Provost for Research for the resolution of continued non-compliance or serious infractions.

C. Institutional Biological Safety Officer (IBSO)

1. Conduct periodic inspections of laboratories to ensure compliance with established containment procedures.
2. Investigate laboratory accidents and report problems, violations and injuries or illnesses associated with biohazardous research activities, to the Institutional Biosafety Committee.
3. Develop and implement emergency plans for handling accidental spills and personnel contamination.
4. Provide advice and assistance to the Institutional Biosafety Committee and Principal Investigators concerning containment procedures and practices, laboratory security, recommended laboratory containment equipment, rules, regulations, and other matters as may be necessary.
5. Provide oversight and assurance that laboratory safety containment equipment is functioning properly including field testing and certification, where appropriate, of all biosafety cabinets.
6. Serve as a member of the Institutional Biosafety Committee.

D. Environmental Health and Safety (EHS)

1. Provide industrial hygiene and safety support for all laboratory operations.
2. Transport and dispose of all infectious waste in compliance with all applicable federal, state, and local ordinances.
3. Assist, as necessary, in the emergency response, cleanup, and decontamination of biological spills and accidents.
4. Administer the University Occupational Health Program.

E. Research and Sponsored Programs (RSP)

1. Provide the necessary liaison between Principal Investigators, the Institutional Biosafety Committee, granting agencies, and regulatory agencies.
2. Serve as the Office of Record for documentation involving the Institutional Biosafety Committee.
3. Provide all necessary documentation, forms, regulatory guidelines and regulations, etc. for Principal Investigators.

F. Laboratory Animal Resources (LAR)

1. Provide appropriate animal husbandry and care that meets or exceeds federal, state, and local requirements and specifications.
2. Ensure that animal housing systems are designed and utilized in a manner that will minimize the potential exposure of other animals or personnel to potentially biohazardous agents.
3. In cooperation with the investigator, the Institutional Biosafety Officer, and the Institutional Biosafety Committee, develop and implement specific standard operational

- procedures, in adherence to the ABSL classification of the agent being used addressing animal care, research procedures, and procedures in case of accident or equipment failure.
4. Ensure that all animal care personnel are adequately trained and aware of the potential risk associated with each agent.
 5. Develop, in cooperation with the institutional Biosafety Officer, emergency plans for handling accidental spills, personnel exposures, unintentional animal exposure, equipment failure, etc.

G. Principal Investigator (PI)

1. Ensure compliance with appropriate National Institute of Health guidelines and all conditions stated in the protocol approved by the Institutional Biosafety Committee.
2. Submit protocol applications for all activities or modifications of activities involving biohazardous materials and obtain approval by the Institutional Biosafety Committee prior to initiation of the activities or modifications.
3. Ensure that all laboratory staff, including students, are trained in the accepted procedures in; laboratory practices, containment methods, disinfectant and disposal practices, and required actions in the event of an accidental spill.
4. Develop a Laboratory Safety Plan, including an emergency action plan for accidents and spills, as an addendum to this manual, when required.
5. Ensure compliance with all shipping requirements for biological agents and toxins.
6. Ensure proper handling and disposal of all infectious wastes as outlined in the WSU *Infectious Waste Management Guide* (see Appendix D).
7. Request immunizations for laboratory personnel when working with biological agents for which there is an effective vaccine available.
8. Maintain all biosafety equipment in appropriate operating condition. Decontaminate laboratory equipment prior to maintenance or disposal.
9. Maintain records of microorganisms and toxins used in the laboratory and biosafety cabinets.

H. Laboratory Staff

1. Conduct no activities under the research protocol until the protocol is approved by the Institutional Biosafety Committee and appropriate training is completed.
2. Follow all procedures and containment methods established for activities conducted.
3. Properly utilize all laboratory protective equipment including proper clothing, personal protective equipment, and containment devices.
4. Report all accidents and spills to the Principal Investigator or the Institutional Biosafety Officer as soon as possible.
5. Report unsafe conditions to the Principal Investigator, the Institutional Biosafety Officer, or the Institutional Biosafety Committee.

SECTION 3 - Regulatory Compliance

- A. Recombinant DNA activities - The NIH *Guidelines for Research Involving Recombinant DNA Molecules* governs all rDNA activities including those exempt by the guidelines.
- B. Non-rDNA activities involving microorganism and exempt rDNA microorganism - Activities involving these agents are not federally regulated but it is the position of the IBC and the IBSO that the procedures and containment levels outlined in CDC publication *Biosafety in Microbiological and Biomedical Laboratories* will govern such activities at Wright State University.
- C. Biological Toxins - These agents are not governed by NIH or CDC regulations or guidelines. Although Material Safety Data Sheets (MSDS) are available for most of these agents, specific exposure levels, to our knowledge, have not been established. EHS will work with the PI to interpret the MSDS and to establish work and disposal procedures which will protect the users of the materials and the environment outside the laboratory.
- D. Blood and Other Body Fluids - OSHA's standard on bloodborne pathogens will govern any activity involving human blood or other potentially infected body fluids. Compliance with this standard is administered by EHS. Information on the university's blood-borne pathogen program can be found in The Wright Way Policy No. 6034, *Occupational/Nonoccupational Exposures to Blood-Borne Pathogens*.
- E. Tuberculosis - OSHA has published guidelines for activities which potentially expose people to tuberculosis pending publication of an OSHA Standard regulating such exposures in the clinical setting. Compliance with the current guideline and the future standard is administered by EHS. Research activities involving *Mycobacterium tuberculosis* will be governed accordingly by NIH or CDC guidelines. Should the OSHA standard apply to the research environment in the future, those requirements will be made available to the PI's.
- F. Chemicals - Chemical usage in educational and research laboratories are governed by OSHA Standard 1910.1450, *Occupational Exposures to Hazardous Chemicals in Laboratories*, and is administered by EHS.
- G. Radioactive Materials and Radiation-Producing Devices - Regulated by the Nuclear Regulatory Commission and the Ohio Bureau of Radiation Protection. The radiation safety program is administered by the Radiation Safety Committee and enforced by the Radiation Safety Officer. The Radiation Safety Manual contains university procedures for using radioactive materials and radiation-producing devices.
- H. Disposal of Infectious Materials - Governed by the Ohio Environmental Protection Agency and administered by EHS. All requirements for managing infectious waste are outlined in the *WSU Infectious Waste Management Guide*. A copy of the Guide is contained in Appendix D.

SECTION 4 - Summary of Biosafety Levels

A. Assignment of Biosafety Levels (BSL) - It is the responsibility of the PI to initially assign the BSL to his/her protocol. This level may be changed either upward or downward during review by the IBC. All parties involved in assigning BSLs will follow the standard levels of 1, 2 or 3 as outlined in either NIH's *Guidelines for Research Involving Recombinant DNA Molecules* or CDC's *Biosafety in Microbiological and Biomedical Laboratories*. The BSLs correspond directly to the class of organism to be used in a protocol. The classes are defined as follows:

Class 1	Organisms not known to cause disease in healthy adults.
Class 2	Associated with human disease, infection transmitted through autoinoculation, ingestion, and mucous membrane exposure.
Class 3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.
Class 4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission.

SECTION 5 - Protocol Submission and Review

- A. Mandatory Submission of Protocol Application - PI's proposing research/academic activities involving microorganisms (including exempt and non-exempt rDNA activities) and biological toxins must complete the university's Biosafety Protocol Application form and submit it to Research and Sponsored Programs (RSP), 122 Allyn Hall for review and approval action prior to initiation of the activity.
- B. Voluntary Submission of Protocol Application - Researchers working with blood or other potentially infectious body fluids, carcinogens, mutagens or teratogens in the absence of microorganisms are requested to complete the university's Biosafety Protocol Application form and submit to Environmental Health and Safety (EHS), 131 Allyn Hall for review. The voluntary submission of this information is of benefit to EHS in their administration of the OSHA programs governing occupational exposures to these agents. The review of these protocol applications will be undertaken by IBSO in cooperation with EHS. There will be no assignment of a biosafety level for these protocols.

C. Protocol Review and Approval

1. rDNA Activities - All protocols involving rDNA activities must follow the requirement of the National Institutes of Health as presented in the latest edition of the NIH Guidelines for Research Involving Recombinant DNA Molecules and all supplements published thereafter in the Federal Register. It is the responsibility of the PI and the IBC to ensure review by the appropriate department of the Public Health Service (NIH and/or FDA) when required under current government guidelines. Non-exempt BSL 1 and 2 and exempt rDNA protocols not requiring review by federal agencies can be approved by the IBC.

Areas to address when rDNA protocols are being prepared for submittal to RSP are:

- a. rDNA Insert
 - (1) Synthetic and associated sequence(s)
 - (2) Potential protein product
 - b. Vector
 - (1) Carrier used to introduce rDNA into the host system that facilitates replication
 - (2) Plasmids, organelles, viruses
 - c. Host
 - (1) Organism in which the rDNA replicates
 - (2) Bacteria, yeast, plant, animal cells
 - d. Containment - Several containment methods are described below:
 - (1) Biological Containment:
 - (a) Limit infectivity of vector or vehicle for specific hosts
 - (b) Limit dissemination and survivability of host and/or vector in the environment
 - (2) Physical Containment:
 - (a) Specifically designed equipment and facilities used to physically contain microbes
 - (b) Limit access to facilities
 - (3) Good Laboratory Practices:
 - (a) Specifically designed practices and procedures used to physically contain microbes
 - (b) Mechanisms for inactivation and disposal of microbes
2. Non-rDNA Protocols Involving Microorganisms or Toxins - IBC approval is required for all protocols involving microorganisms. Protocols involving biological toxins in the absence of microorganisms will be under the review authority of the IBSO, but will not be assigned a biosafety level.

SECTION 6 - Training

All IBC members, PI's, and laboratory staff members conducting activities involving microorganisms or biotoxins, are required to receive training in biosafety, regardless of the level of activity they propose to use (BSL1, BSL2, or BSL3). This commitment can be met by attending the training sessions given by members of the IBC or viewing the video training film developed by the IBC. Record of attendance will be maintained by the IBSO.

Additionally, the PI is responsible for the development and administering of training to their laboratory staff members and students. This training shall address biosafety and laboratory safety relative to the activities on-going in the laboratory. Training should include, but not necessarily be limited to, procedures and techniques, laboratory safety rules, emergency response, spill containment and cleanup, and instructions on the operating parameters and procedures for use of laboratory equipment (chemical fume hood, biosafety cabinets, autoclaves, centrifuge, etc.). **Some equipment, when used improperly, can give less than desired results and may even result in accidents with severe injury to the user.**

Manufacturer's Operating Manuals are normally an excellent source of information on the proper and safe use of equipment. Generalized information on laboratory procedures, and safe use of equipment is available in NIH's *Laboratory Safety Monograph* and NRC's *Biosafety in the Laboratory*. Both publications are available for loan from EHS at 131 Allyn Hall.

Training must be given to all new laboratory employees or students and all members of the laboratory staff should receive annual refresher training. Record of attendance and the training provided should be maintained by the PI. Records are to be kept for a minimum of three (3) years. A variety of biosafety courses are available through EHS which can assist the PI in meeting training requirements.

PI's will also ensure that everyone involved in their laboratory operations also participate in other training requirements relative to health and safety as administered by EHS and LAR. Examples of this training would include, but not necessarily be limited to, Blood-Borne Pathogens, Hazards Communications, Chemical Hygiene Plan, Care and Usage of Laboratory Animals, etc.

SECTION 7 - Containment

- A. Definition - Containment is the use of safe methods for managing infectious agents in the laboratory environment where they are to be handled or maintained.
- B. Purpose - to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

C. Types of Containment

1. Primary (p): methods to protect the internal laboratory environment, e.g., microbiological techniques and appropriate safety equipment.
2. Secondary (s): methods to protect the environment external to the laboratory, e.g., facility design and operational practices.

D. Required Levels of Containment - In general, the requirements listed below are considered minimum methods for containment at the appropriate BSL. The PI is responsible for referring to NIH's *Guidelines for Research Involving Recombinant DNA Molecules* or CDC's *Biosafety in Microbiological and Biomedical Laboratories* for specific containment requirements. The PI is also responsible for ensuring that secondary methods, e.g., facility design features or special practices are incorporated into the protocol when primary or standard practices are not sufficient for containment. The IBC, in its review of protocols, will give serious consideration for the need of additional containment.

1. BSL 1: Laboratory work involves the use of organisms not known to cause disease in healthy adults and present a minimal potential hazard to laboratory personnel and the environment. Special containment equipment is not required and work is generally conducted on the open bench top.

Laboratory Practices include:

- Standard Microbiological Practices (p)
- Required Personal Protective Equipment (p)
- Open bench top sink required (s)

2. BSL 2: Laboratory work involves the use of agents that present a moderate potential hazard to laboratory personnel and the environment. With the added hazards, access to the laboratory is limited when work is being conducted and training is given to the laboratory personnel on the proper handling and the hazards of the organisms being used. A procedural guide for handling of agents may be needed. A biological safety cabinet is used for material manipulations where there is a potential for aerosols being generated.

Laboratory Practices include:

- BSL 1 practices (p) plus:
- Limited access
- Biohazard warning signs
- "Sharps" (needles, scalpel, knives, etc) precautions
- Lab Safety Plan as addendum to this manual when required
- Class I or II biosafety cabinet or other physical containment device (p) used for all manipulations of the agents that cause splashes or aerosols of infectious materials.

- Laboratory coats, gloves, face protection (p)
- Autoclave (s)

3. BSL 3: Laboratory work involving indigenous or exotic agents that have the potential to cause serious illness or are lethal if inhaled. Access to the laboratory work area is restricted. The laboratory personnel are given specific training regarding proper handling and potential hazards of the agent that is being used. At this level of containment there is a specific need for administrative and procedural guidelines for operations.

Laboratory Practices include:

- BSL 2 (p) practices plus
- Controlled access
- Decontamination of waste before removal from laboratory
- Decontamination of lab clothing before laundering
- Baseline serum (when appropriate, considering the agent(s) handled) will be taken and archived.
- Class I or II biosafety cabinet or other physical containment devices (p) used for all manipulations of agents
- Protective lab clothing, gloves, eye, face protection, respiratory protection, as needed (p)
- Physical separation from access corridor (s)
- Self closing, double door access (s)
- Exhausted air not recirculated (s)
- Negative airflow into laboratory (s)

SECTION 8 - Use of Animals

The requirements for the use of animals with biohazardous agents are similar to, but not identical to, the requirements for the use of the same agent in laboratory situations. The PI, in conjunction with the Director of LAR, is responsible for determining the appropriate Animal Biosafety Level (ABSL) for the specific agent being utilized. NIH's *Guidelines for Research Involving Recombinant DNA Molecules* or CDC's *Biosafety in Microbiological and Biomedical Laboratories* should be consulted for the appropriate classification and requirements for the use of the proposed agent.

The LAR has facilities appropriate for the handling of ABSL 1 through 3 agents, if appropriate caging is available. Three dedicated negative pressure animal rooms with 100 percent exhaust are available, as well as a Class IIA biological safety cabinet for animal handling and care. Animal care personnel in the LAR are trained in the handling and care of animals infected with biohazardous agents. Specific requirements for each proposed biohazardous agent must be delineated and appropriate safety precautions developed prior to the initiation of any experiment

utilizing the agent. A meeting between the PI and the Director of LAR is required prior to initiation and preferably during the design of the experiment.

The basic requirements for each ABSL are outlined below:

1. ABSL 1 -
 - Restricted access to the animal facility. Only personnel advised of the potential hazards are permitted access.
 - Personnel at increased risk to infection are not permitted access.
 - Standard microbiological practices (wash hands, no eating, drinking, smoking, handling contact lenses, applying cosmetics, or storing of human food in animal areas). Personnel wearing contact lenses should wear goggles or face shields.
 - Appropriate laboratory apparel is required (laboratory coats, gowns, scrubs, etc.)
 - Animal wastes are appropriately decontaminated and the cages cleaned and decontaminated, preferably by washing in a mechanical cage washer with a final rinse of 180 degrees F or higher.
 - Adopt the Institutional Biosafety Manual as a supplement to NIH's *Guidelines for Research Involving Recombinant DNA Molecules* and CDC's *Biosafety in Microbiological and Biomedical Laboratories*.

2. ABSL 2 -
 - ABSL 1 practices plus:
 - Biohazard signs, including the universal biohazard symbol, are posted where special entry requirements are necessary. The signs shall include identification of the agent, identification of responsible person(s) (including telephone number(s)), and the special requirements necessary for entry.
 - "Sharps" precautions are followed.
 - Class I or II biological safety cabinets are utilized for all procedures where the potential for aerosolization of infectious material or bedding may occur.
 - Animals are housed in cages designed to minimize the potential spread of aerosols.
 - Appropriate laboratory clothing is worn while in the animal area and the clothing is removed before leaving the animal area.
 - As appropriate, laboratory personnel receive appropriate immunizations and serum sampling.
 - Animal wastes, bedding, and cages are appropriately decontaminated, preferably by autoclaving, prior to cleaning.
 - Only animals actually involved in the work being performed are permitted in the animal room.
 - Develop laboratory safety plan as an addendum to the Institutional Biosafety Manual, when required.

3. ABSL 3 -
 - ABSL 1 and ABSL 2 practices plus:
 - Cage(s) are autoclaved or thoroughly decontaminated before bedding and animal wastes are removed and the cage(s) cleaned.
 - Personnel receive appropriate vaccination where available and baseline serum samples are collected and appropriately archived.

SECTION 9 - Laboratory Safety Manual

Where the hazard associated with a particular agent cannot be controlled using the guidance of this manual and those of the NIH's *Guidelines for Research Involving Recombinant DNA Molecules* and/or CDC's *Biosafety in Microbiological and Biomedical Laboratories* or by requirements levied by the IBC, the PI will develop a laboratory safety manual for the protocol in question. The laboratory manual will serve as an addendum to this manual. Where required, special practices will be included in the manual as well as emergency response procedures for handling spills. Staff members and students must read and understand this manual as well as the laboratory safety manual.

SECTION 10 - BSL 3 Facility: Tests and Certifications

The minimum certification requirements necessary before operations in a BSL3 facility can begin are as follows:

1. Access Control - May be a single laboratory module or a complex of modules within a building or an entire building. The facility must be separated by a controlled access zone from areas open to the public and other laboratory personnel who do not work in the BSL3 facility.
2. Penetration Seals -The openings in walls, floors and ceiling through which utility services and air ducts penetrate must be sealed to permit space decontamination
3. Directional Airflow - The ventilation system supporting the containment facility must be capable of controlling air movement. The direction of airflow is to be from areas of lower contamination potential to areas of higher contamination potential. The system is balanced so that there is infiltration of air into the facility from the adjacent corridors. The infiltration rate should be at least 50 cubic feet per minute. Certification of directional air movement should be accomplished at least quarterly or whenever it is suspected that a deficiency exists. The quarterly evaluation will be conducted by the PI. Annual verification will be completed by EHS. EHS, in conjunction with the PI, will develop procedures for the directional air movement tests.
4. Exhaust Air Ducts - No cross connection between supply air duct and exhaust air ducts is permitted.

5. Steam and Ethylene Oxide Sterilizers - Tests which demonstrate the performance of steam and ethylene oxide sterilizers must be accomplished and documented before operations begin in the facility and periodically thereafter. Procedures for these tests as outlined in NIH's Laboratory Safety Monograph, are listed in Appendix C. Tests should be done at a minimum of every six months and may be more frequent depending on the nature of the activities being conducted. Testing should also be accomplished following all major maintenance. It is the PI's responsibility to perform the required testing of this equipment.

SECTION 11 - Biosafety Cabinets

- A. Selection of Appropriate Cabinet - It is recommended that Class II biosafety cabinets be utilized for all activities involving Class 2 or 3 microorganisms. Class I cabinets have definite limitations and are only manufactured in limited numbers. As to which make or model, the PI needs to consider the relative hazards of the infectious agent, potential for creating infectious aerosols, costs and anticipated future protocols. **It is strongly recommended that PI's purchase only National Sanitation Foundation (NSF) certified biosafety cabinets.** The IBSO and EHS, as well as manufacturer's representatives, can provide advice on the selection of the appropriate cabinet.
- B. Field Certification of Cabinets - NSF listed biosafety cabinets are to be field certified under the following conditions.
1. Upon initial installation of the cabinet
 2. Annually thereafter
 3. When moved or relocated within and/or outside the lab

EHS will also test but cannot certify non-NSF units belonging to Wright State University.
There is no cost for EHS's services.

Certification is also required following major maintenance on a biocabinet or replacement of the HEPA filters. EHS does not perform this type of maintenance on cabinets nor will they certify cabinets following completion of such work. See paragraph D below, Repair and Maintenance.

- C. Use of Chemical/Radioactive Agents - It is recommended that the PI confer with the IBSO and EHS before using chemical or radioactive agents in a biosafety cabinet. **Volatile chemicals, carcinogens and radioactive materials cannot be used in Class I, IIA and IIB3 biosafety cabinets.**
- D. Repair and Maintenance - It is recommended that all repairs or maintenance of biosafety cabinets be accomplished by manufacturer's service representatives. This includes the replacement of HEPA filters. Minor repairs such as replacement of light bulbs, small electrical problems, etc. might be accomplished by Physical Plant personnel following submittal of a work request. Decontamination of the HEPA filters may be required under some conditions when certifying a cabinet or when filters require replacement. This

illustrates the importance of maintaining records of organisms and toxins used in the biosafety cabinet. If one cannot prove that only non-pathogenic materials have been used, the service representative will most certainly require that the cabinet be decontaminated.

Biosafety cabinets are not to be disassembled for moving or for filter replacement until decontamination (if required) is completed and approval is given by the IBSO and/or EHS. Recertification of the cabinet following major maintenance and HEPA filter replacement is to be accomplished by a manufacturer's service representative and should be included in the Statement of Work. **THIS HOLDS THE CONTRACTOR ACCOUNTABLE FOR HIS WORK PERFORMANCE.** All costs associated with maintenance, filter replacement, and recertification is at the expense of the using department. The PI may request the EHS staff member, certified to test biosafety cabinets, to observe the contractor during certification procedures.

- E. Training on Use of Biosafety Cabinet - EHS can assist in providing training to laboratory personnel in the proper use of biosafety cabinets. Videos are also available which address proper utilization of biosafety cabinets.

SECTION 12 - Decontamination and Disposal of Infectious Materials

- A. Laundering of Contaminated Clothing - A laundry facility is provided in 134 Biological Sciences II for laundering of laboratory clothing. There is no cost to use this facility. Keys are available in the departmental offices for this room. It is recommended that all laboratory clothing be laundered at this facility and not taken home. Clothing potentially or known to be contaminated with infectious materials must be decontaminated, preferably by autoclaving, before laundering. Clothing contaminated with radioactive material may not be laundered in this facility. For assistance in decontaminating clothing or handling clothing contaminated with radiation, please contact the IBSO or the Radiation Safety Officer (RSO). Clothing contaminated with a flammable liquid must be aired completely before it can be laundered and dried.
- B. Disinfectant Guidelines - The choice of the appropriate disinfectant is critical in any experiment involving a biohazardous agent. A number of different classes of disinfectants are available including phenols, quaternary ammonium compounds, chlorhexidine compounds, halogen compounds, alcohols, aldehydes, etc. The decision as to the most appropriate agent requires a knowledge of both the organism's susceptibility to the agents and the type of substrate that will require decontamination. Agents offering the lowest potential for personnel or environmental toxicity should be used whenever possible.

Each laboratory must prepare a specific protocol for handling the decontamination of work surfaces, equipment, and spills. The CDC recommends the use of a 1:10 dilution of household bleach (5,250 ppm sodium hypochlorite final dilution) in neutral water with a 20 minute contact time for the decontamination of blood spills. Other disinfectant solutions may be more appropriate for other situations. Appendix E, Guidelines for the Use of

Disinfectants, contains additional information for selecting the most appropriate disinfectant. The IBSO and the Director, LAR are also available to assist investigators in developing appropriate disinfectant and decontamination procedures.

- C. Disposal Practices - Disposal of all potentially or known infectious materials will be accomplished according to procedures contained in Appendix D, *WSU Infectious Waste Management Guide*. Laboratory personnel may, at their discretion, decontaminate infectious material within their laboratory facilities, however, the waste must still be handled and disposed of by procedures outlined in the *WSU Infectious Waste Management Guide*. **There are no exceptions to this policy.**

SECTION 13 - Reporting of Exposure Incidents and Spills

- A. Exposure Incidents - PI's are required to report all incidents which result in a spill of infectious material and/or an exposure to individuals which could result in illness or disease. For infectious materials both the IBSO and EHS must be notified. Reporting of the incident to EHS will be accomplished in accordance with procedures outlined in Wright Way Policy 6032, *Reporting of Injuries and Illnesses*. If the incident involves blood or other potentially infectious body fluids, the requirements outlined in Wright Way Policy 6034, *Occupational/Nonoccupational Exposure to Blood-Borne Pathogens*, must be followed. If injuries or illness results from the incident, emergency care should be obtained as outlined in Wright Way Policy 6031, *Emergency Care for Injuries and Illnesses*.
- B. Handling Spills of Infectious Materials - Containment and cleanup procedures for spills of infectious materials are contained in the *WSU Infectious Waste Management Guide*. A copy of the guide is contained in Appendix D of this manual.

SECTION 14 - Packaging and Shipment of Biological Materials

The importation or shipment of biological materials are governed by the Center for Disease Control. Information required of the PI for receipt or shipment is contained on pages 148-149 of CDC's *Biosafety in Microbiological and Biomedical Laboratories*.

SECTION 15 - Maintenance/Repair and Disposition of Equipment

Maintenance and Repair Activities:

It is the PI's responsibility to ensure that equipment and work surfaces which are contaminated or potentially contaminated with chemical, infectious and/or radiological materials are properly decontaminated before clearance is given for any maintenance/repair or custodial activities. This includes, but may not be limited to the activities of Physical Plant (maintenance/repair and custodial), Instrument Shop, Electronic Shop, contract personnel or factory representatives. **NOTE: Physical Plant has limited capabilities for the maintenance of laboratory equipment.**

Appendix E provides insight into the method of choice for disinfecting various equipment and surfaces.

Biosafety Cabinets:

The methodology presented in Appendix E can be applied to the inside working surface and the exterior surface of biosafety cabinets under normal use conditions. They do not apply to certain conditions of relocation and maintenance, as listed below, when it is deemed necessary that the entire internal components of the cabinet be decontaminated. **For these situations, the biosafety cabinet will be decontaminated only by individuals that are properly trained and qualified to do so. The university does not have anyone qualified to perform these decontamination procedures on biosafety cabinets. THERE ARE NO EXCEPTIONS TO THIS RULE.** The Institutional Biosafety Officer and the Sr. Industrial Hygienist qualified to test and certify biosafety cabinets (see Appendix B) must be notified when any of the below conditions exist and a biosafety cabinet requires decontamination by a certified individual. Environmental Health and Safety can provide information as to the availability of certified individuals in the local area. Decontamination, required under the following conditions, will be applicable for all biosafety cabinets used for BSL 2 and 3 activities and when the PI cannot provide documentation that all previous usage involved only BSL 1 or non-microbiological activities:

- Release of the cabinet for unrestricted use.
- Moving the cabinet to another laboratory or area of use.
- Repair work requiring access to the sealed plenum.
- Replacement of HEPA filters.
- Service or replacement of the cabinet circulation fan or components.
- Release of the cabinet to ESPM for resale or salvage.
- Prior to annual field certification at the discretion of the certifier.

NOTE: The above conditions require thorough decontamination using paraformaldehyde gas or other decontamination methods approved by NSF.

ESPM Procedures:

The PI should refer to Wright State Policy 5403.6(f) for procedures to follow in disposing of laboratory equipment through ESPM. Environmental Health and Safety will be asked, by ESPM personnel, to verify that the equipment has been decontaminated and that there is no residual chemical, infectious and/or radiological materials.

APPENDIX A REFERENCES

1. National Institute of Health publication, Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), January 1996 or as periodically updated
2. CDC publication, Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition
3. NIH publication, Laboratory Safety Monograph, A Supplement to NIH Guidelines for Recombinant DNA Research, January 1979
4. Environmental Health and Safety, Infectious Waste Management Guide, January 1998 (revised)
5. Environmental Health and Safety, Chemical Hygiene Plan, September 1993
6. The Wright Way Policy 6031, Emergency Care for Injuries and Illnesses, September 1994
7. The Wright Way Policy 6032, Reporting of Injuries and Illnesses, April 1994
8. The Wright Way Policy 6034, Occupational/Nonoccupational Exposures to Blood-Borne Pathogens, October 1994
9. Occupational Safety and Health Act (OSHAct), Part 1910, Subpart Z, Section 1910.1030, Blood-borne Pathogens, December 1991
10. National Research Council, Biosafety in the Laboratory, National Academic Press, Washington DC (1989)

**APPENDIX B
 DIRECTORY OF WSU CONTACTS FOR
 BIOSAFETY INQUIRIES**

TITLE	NAME	LOCATION	EXT.
Chairperson, Institutional Biosafety Committee	Dawn Wooley, Ph.D	143 Biological Sciences II	4993
RSP Representative	Ms. Ellen Reinsch Friese	201J Allyn Hall	2709
Director of LAR	Gregory Boivin, DVM	053 Health Sciences	2792
Institutional Biological Safety Officer	Kimberly Morris, CHMM	104 Health Sciences	2623
Infectious Waste	Bill Palmer, CHMM	047 Biological Sciences II	3788
Biological Safety Cabinet Certification	Greg Merkle	047 Biological Sciences II	2215 2217

**APPENDIX C
PROCEDURES FOR CERTIFICATION
OF
STEAM AND ETHYLENE OXIDE STERILIZERS**

A. Equipment and Materials Required:

1. Spore strips containing both *Bacillus subtilis* var. *niger* and *Bacillus stearothermophilus*. (Amsco Spordi or equal)

Employ separate spore strips with an average certified population of 10,000 *B. stearothermophilus* and 1,000,000 *B. subtilis* spores, adjusted to the following resistance data.

Test Organisms	Sterilization Medium	Exposure Time & Temperature (F)	
		<u>Survives</u>	<u>Killed</u>
<i>Bacillus stearothermophilus</i>	Steam	250 deg; 5 min	250 deg; 13 min
<i>Bacillus subtilis</i> var. <i>niger</i>	EtO	15 min	1 hour, 45 min

2. Temperature indicator with remote probes.
3. Hand Towels, 16 X 24 inches.
4. Stainless steel pan approximately 12" X 18" X 2" deep.
5. Supporting laboratory equipment (incubator, refrigerator, culture media, miscellaneous glassware).

B. Steam Sterilizer Test Procedure:

1. Fold in half three hand towels and stack them in the stainless steel pan. Place one test spore strip into the fold of the top and bottom towels. Do not remove the spore strips from their glassine envelopes.
2. Place the temperature probe into the fold of the center towel with the lead extended over the lid of the pan. Place a second probe in the sterilizer drain. Position the pan in the rear center of the sterilizer away from the steam inlet. Pass the temperature lead out of the sterilizer chamber and connect to the recorder.

3. Close the door, taking care not to cut the probe lead wires.
4. Operate the sterilizer in accordance with the manufacturer's instructions. The cycle (time and temperature of exposure) shall be set as follows:
 - a. Set the minimum time that is required to kill the test spore strips located in the test pan. Use approximately the "kill" time and temperature established above once the temperature indicator located in the test pan reaches 250 deg F or 121 deg C.
5. Record the temperature readings from the indicator (probe leads inside the sterilizer at three minute intervals. Simultaneously, record the chamber temperature, chamber pressure and jacket pressure as shown by the sterilizer indicator.
6. Upon completion of the cycle, rapidly exhaust the chamber and then remove the test spore strips from the sterilizer.
7. Aseptically remove all test spore strips and two unheated control strips from their glassine envelopes with sterile forceps and place in previously prepared 12 X 150 mm tubes containing 10 mL of sterile Trypticase Soy Broth.
8. Incubate one set of test and control tubes for seven days at 55 deg C for *B. stearothermophilus* detection. Incubate the second set of test tubes and control tubes for seven days at 37 deg C for *B. subtilis* var. *niger* detection.
9. All test organisms on each test strip must be killed (i.e., no growth may be visually present after incubation). The control strips must show positive results after incubation.
10. In the event of test failure, corrective action (e.g., readjustment of steam sterilizer time/temperature) must be undertaken. The test must be repeated to ensure the adjustments were successful.

C. Ethylene Oxide Sterilizer Test Procedures:

1. Fold in half three hand towels and stack them in the stainless steel pan. Place one test spore strip into the fold of the top and bottom towels. Do not remove the spore strips from their glassine envelopes.
2. Position the pan in the rear center of the sterilizer away from the gas inlet.
3. Operate the sterilizer in accordance with the manufacturer's instructions.
4. Upon completion of the gas cycle, rapidly exhaust the chamber and then remove the test spore strips from the sterilizer.

5. Aseptically remove all test spore strips and two unexposed control strips from their glassine envelopes with sterile forceps and place in previously prepared 12 X 150 mm tubes containing sterile Trypticase Soy Broth.
6. Incubate one set of test and control tubes for seven days at 55 deg C for *B. stearothermophilus* detection. Incubate the second set of test and control tubes for seven days at 37 deg C for *B. subtilis* var. *niger*.
7. All test organisms on each test strip must be killed (i.e., no growth may be visually present after incubation). The control strip must show positive results after incubation.
8. In the event of test failure, corrective action (readjustment of gas concentration and/or exposure time) must be undertaken. The test must then be repeated to ensure that the adjustment was successful.

TEST CRITERION: ALL SPORES ON EACH TEST STRIP MUST BE KILLED.