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 Plant, 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, Section1910.1030, *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)**	
		Survives	**Killed**
Bacillus stearothermophilus	Steam	250 deg; 5 min	250 deg; 13 min
Bacillus subtilis var. *niger*	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.

APPENDIX D
INFECTIOUS WASTE MANAGEMENT GUIDE

TABLE OF CONTENTS

I. INTRODUCTION:

On May 1, 1990, the Ohio Environmental Protection Agency (OEPA) amended Chapter 3745 of the Ohio Administrative Code (OAC). These and subsequent amendments regulate the generation, management, and disposal of infectious waste.

For Wright State University (WSU) to comply with these regulations, individual generators of infectious waste at WSU and WSU affiliated facilities will have to follow the procedures outlined in this guide as a means of managing their infectious waste. This guide should be reviewed periodically by all personnel responsible for areas where infectious waste is generated. The guide MUST also be distributed to, and reviewed by, all personnel working in areas where infectious waste is generated. The guide will be updated as the OEPA revises the regulations or as WSU changes procedures.

The following appendices are included at the end of this guide to assist the individual generators of infectious waste:

Appendix I - OEPA Definition of Infectious Waste

Appendix II - WSU Infectious Waste Spill Containment and Cleanup Procedures

II. SUMMARY OF REGULATIONS:

In addition to defining infectious waste, the amendments of OAC Chapter 3745 also provide specific packaging, handling, treatment and transportation requirements, as well as procedures for infectious waste spill containment and cleanup.

A. What is Infectious Waste?

Infectious waste is waste resulting from the work with infectious or zoonotic agents. OEPA's definition of infectious waste, infectious agent, and zoonotic agent can be found in Appendix I of this guide.

B. Infectious Waste Packaging and Handling Requirements:

All individual generators of infectious waste must segregate infectious waste from other waste at the point of generation. Infectious waste, other than sharps (i.e., hypodermic needles, syringes, scalpel blades, and glass articles that have been broken) or liquids, shall be accumulated in red or other colored plastic bags labeled with the international biohazard symbol. Bags containing infectious waste that are being transported off the premises where generated shall be placed inside a second sealed bag or fully enclosed, rigid, sturdy container. All sharp infectious waste shall be accumulated only in containers specifically designed and

manufactured for the management and/or disposal of "sharps" and be labeled with the international biohazard symbol and with the word "SHARPS". All unused or non-infectious discarded hypodermic needles, syringes, scalpel blades, and glass articles that have been broken shall be accumulated in rigid, puncture resistant, leak resistant containers closed tightly to prevent loss of content and be labeled as "SHARPS". Containers consisting solely of unused or non-infectious sharps are not required to be labeled with the international biohazard symbol. Liquid or semiliquid infectious waste consisting of blood, blood products, body fluids, and excreta shall be disposed of as described in paragraph C below. Liquid infectious cultures can be chemically treated and disposed of as described in Section III, A.3 on page 6. No infectious waste shall be compacted or ground when packaged.

All packaged infectious waste shall be stored in a manner that maintains the integrity of the packaging using refrigeration or freezing when necessary. All storage areas must be limited to authorized personnel or labeled as an infectious waste storage area.

C. Infectious Waste Treatment Requirements:

All infectious waste must be treated prior to disposal. Treatment can be performed on the premises where the infectious waste is generated or the infectious waste can be shipped off-site to a permitted infectious waste treatment facility. Wherever the infectious waste is treated, it must be treated by methods approved by the OEPA.

OEPA approved methods for the treatment of infectious waste includes incineration, autoclaving, chemical treatment with sodium hypochlorite solution (used for cultures only) and other methods specifically approved by the OEPA. In order to comply with the infectious waste treatment regulations, a treatment facility must meet operational and quality control requirements. For incineration and autoclaving, this includes, but is not limited to:

- Specific design criteria
- Temperature and residence time requirements
- Temperature and pressure (for autoclaving) monitoring
- Treatment area drainage requirements
- Scheduled calibration and testing
- Loading requirements
- Personnel training
- Documentation and records retention requirements
- Contingency plans for alternate treatment

Chemical treatment utilizing sodium hypochlorite (bleach) is a method approved for the treatment of cultures only.

Individual generators, who generate liquid or semiliquid infectious waste consisting of blood, blood products, body fluids, and excreta may discharge this material to a properly licensed sanitary sewer without prior treatment. This is the only type of infectious waste that can be disposed of without first treating it to render it non-infectious. All other infectious waste must be properly treated before being subjected to the general waste stream as ordinary solid waste.

D. Infectious Waste Transportation Requirements:

All infectious waste, even after it has been treated, which is transported off-site for treatment or disposal must comply with the transportation and shipping paper requirements of OAC Chapter 3745. For transporters who transport untreated infectious waste, these regulations include requirements for registration, labeling, packaging, vehicle design, refrigeration, holding time, disinfecting, spill containment, and cleanup. However, transporters need only comply with the shipping paper requirements if transporting treated infectious waste.

Generators of infectious waste must also comply with shipping paper requirements. There are two different types of shipping papers. The generator must assure that one of the two types accompanies every shipment of infectious waste that is transported off the generator's premises. The OEPA has designed treatment shipping papers which must accompany shipments of infectious waste to an off-site treatment facility and disposal shipping papers which must accompany treated infectious waste to an off-site disposal facility (i.e., landfill). Both shipping papers consist of multi-copy carbonless paper that is designed to track the waste from "cradle-to-grave" and assure the generator that the waste has reached its intended destination.

E. Infectious Waste Spill Cleanup and Containment Requirements:

If a spill or accidental release of infectious waste occurs during the process of generating, packaging, handling, treating or transporting infectious waste, the person responsible for the spill/release must comply with spill cleanup, containment, and reporting requirements. These requirements differ slightly for generators, transporters, and treatment facilities.

Generators must develop and implement a written spill containment and cleanup procedure that must be readily available to all persons likely to handle infectious waste.

Transporters must also develop and implement a spill cleanup and containment plan, as well as train all employees in the implementation of the plan.

Transporters must also carry spill containment and cleanup kits consisting of specific cleanup material.

Infectious waste treatment facilities and generators who treat their own waste by incineration or autoclaving must develop and implement a written spill cleanup and containment plan and provide training to all individuals who handle infectious waste. A spill cleanup and containment kit consisting of specific cleanup material must be kept in the vicinity of any storage, loading, unloading, decontamination, and treatment areas.

THIS SUMMARY IS INTENDED AS AN OVERVIEW OF THE INFECTIOUS WASTE REQUIREMENTS. THESE REGULATIONS APPLY TO GENERATORS OF FIFTY POUNDS OR MORE OF INFECTIOUS WASTE PER MONTH. GENERATORS OF LESS THAN FIFTY POUNDS PER MONTH ARE SUBJECT TO LESS STRINGENT REQUIREMENTS. TO OBTAIN A SET OF THE INFECTIOUS WASTE REGULATIONS, CONTACT THE DEPARTMENT OF ENVIRONMENTAL HEALTH AND SAFETY AT EXT. 3788 OR THE OEPA AT (614) 644-2621.

III. WRIGHT STATE UNIVERSITY INFECTIOUS WASTE STRATEGY:

The regulations implemented by the OEPA governing the generation, management, and disposal of infectious waste create an impact on how WSU research, teaching, and health professionals handle waste contaminated or potentially contaminated with infectious agents. Depending on the time of year, WSU and WSU affiliated facilities generate between 300 and 900 pounds of infectious waste per month. This makes WSU a large quantity generator and subjects the university to the regulations for large quantity generators, as summarized in Section II of this guide.

As a result of the regulation, a decision was made to transport WSU's infectious waste off-site for treatment and disposal as opposed to treatment of infectious waste on-site (other than chemical treatment of cultures). This decision leaves WSU with the task of complying with the infectious waste generator requirements only, instead of both generator and treatment facility requirements. The University Task Force on Toxins and Infectious Material made the decision based on the costs and man-hours required to comply with treatment facility requirements (i.e., autoclave room renovation, calibration and testing, employee training, paperwork, etc.) versus the cost to transport and treat infectious waste off-site.

As a result of this decision, WSU individual generators of infectious waste are **NOT** permitted to autoclave or incinerate infectious waste and have it disposed of as ordinary trash. Autoclaves can be used for disinfection and sterilization purposes (i.e., for glassware, equipment) and for the treatment of waste not meeting the OEPA definition of infectious waste. Waste that does not meet the OEPA definition of infectious waste but requires autoclave treatment by another agency (i.e., National Institute of Health) shall be autoclaved in bags not labeled with the international biohazard symbol. Infectious waste will be accumulated in appropriate packages (supplied by the infectious waste contractor) which will be picked up by Environmental Health and Safety personnel for eventual transport off-site and treatment at a licensed facility prior to disposal.

Although infectious waste is not permitted to be treated via autoclaving or incineration and disposed as ordinary solid waste at WSU, on-site chemical treatment of infectious waste cultures (in liquid form only) using bleach is permitted. Also, untreated liquid or semiliquid infectious waste consisting of blood, blood products, body fluids, and excreta may be disposed of into the sanitary sewer system without prior treatment.

A. Procedures for WSU Infectious Waste Generators:

WSU personnel who generate infectious waste shall follow the following steps.

1. All WSU personnel who generate infectious waste must notify the Department of Environmental Health and Safety (EHS). To determine if you generate infectious waste, refer to the infectious waste definitions in Appendix I of this guide. Any infectious waste meeting the definition of hazardous waste due to its chemical properties or which is also radioactive must be managed differently than regular infectious waste. **Any WSU personnel who generates chemically hazardous or radioactive infectious waste must contact EHS to determine proper treatment and disposal procedures.**

2. As a generator, you shall segregate infectious waste from other waste at the point of generation.

3. If you generate cultures (in liquid form), which when intended for disposal meet the definition of infectious waste, you can treat the cultures using approved chemical treatment procedures. The approved chemical treatment procedures are as follows:

    a. Only cultures in liquid form can be chemically treated;

    b. The approved chemical treatment solution shall contain, volume per volume, at least fifteen percent sodium hypochlorite (household grade bleach). The solution shall be at least fifteen percent bleach not fifteen percent sodium hypochlorite which is the active ingredient in bleach;

    c. All cultures shall be submerged or otherwise in complete contact with the chemical treatment solution for a minimum of twenty minutes;

    d. Cultures of infectious agents that are recommended by the Centers for Disease Control to be handled in accordance with biosafety level 3 or 4 practices shall not be treated by these chemical treatment procedures;

    e. The chemical treatment solution shall be mixed immediately prior to use and discarded after use; and

f. All waste cultures, which have been chemically treated, can be released to the sanitary sewer.

4. Untreated liquid or semiliquid infectious waste consisting of blood, blood products, body fluids, and excreta, may be disposed into the sanitary sewer system without prior treatment.

5. If you generate infectious waste other than liquid or semiliquid waste consisting of blood, blood products, body fluids, excreta, or liquid cultures, EHS will supply you with a box to accumulate your infectious waste. The individual generator must supply containers for accumulating "sharps" waste (i.e., hypodermic needles and syringes, scalpel blades, used or unused, and infectious waste glass articles that have broken).

6. All "sharps" infectious waste must be accumulated in approved containers and must be labeled with the international biohazard symbol or the words "Infectious Waste" and with the words "SHARPS". Refer to Appendix III Wright State University Sharps Management Policy to determine the proper method for managing sharps waste.

7. All infectious waste, other than liquid waste, must be accumulated in boxes supplied by EHS. These boxes are supplied by the infectious waste disposal contractor and meet all specifications as required by the Ohio EPA. No other containers shall be used unless first approved by EHS. When using the boxes supplied by EHS, the following restrictions apply:

   a. All waste must be bagged or properly containerized before being placed in a box.

   b. No freestanding liquid shall be poured into a box and no containers of liquid shall be placed in a box without prior approval from EHS.

   c. Absolutely no chemically hazardous or radioactive waste, regardless of its infectious nature, shall be disposed of in a box.

   d. Do not fill a box greater than its maximum weight capacity. The maximum capacity of the large box is 50 lbs. and the small box is 40 lbs.

   e. A full box must have its interior liner bag taped closed and the box properly sealed before it can be picked up.

8. Once a container of infectious waste becomes full, contact EHS at ext. 2215 or 3788, to schedule a pickup. EHS will supply you with a new box. If you generate an infectious waste, which you cannot keep in a nonputrescent state prior to a box becoming full, notify EHS and immediate arrangements for a pickup will be made.

9. All labs and rooms that are accumulating infectious waste must be labeled with the international biohazard symbol at all points of access. These labels will be supplied by EHS.

10. In the event of an accidental spill or release of infectious waste, the person or persons responsible for the spill must instigate containment and cleanup. The WSU Infectious Waste Spill Containment and Cleanup Procedure will be followed. These procedures are provided in Appendix II of this guide. These procedures must be readily available to all personnel involved with the generation and/or handling of infectious waste. All personnel must periodically review and become familiar with the Spill Containment and Cleanup Procedure.

All personnel who generate infectious waste shall follow these steps. By following these procedures Wright State University can maintain compliance in a systematic manner with the Ohio EPA infectious waste regulations. All personnel involved in the generation of infectious waste shall become familiar with the procedures set forth in this guide. If anyone has ideas that may enhance WSU's ability to comply with these regulations, please contact EHS. Input from people who are directly regulated makes compliance much easier and procedures more effective. For any questions, concerns, or input, the Department of Environmental Health and Safety can be reached at ext. 3788.

Appendix E
Guidelines for Disinfection or Sterilization – Infection Control in Biosafety Laboratories

Cleaning, Decontamination, Disinfection, Sanitization and Sterilization

The Purpose of Decontamination, Disinfection and Sterilization

Good housekeeping in a research laboratory should be a principle of operation at all times. Failure to clean up after a procedure, to properly clean used glassware, to clean up after spills or even to have routine cleaning of the laboratory floors by custodial personnel on a scheduled basis can all contribute to the incidental inoculation of research material and of the unsuspecting laboratory worker. General housekeeping of the facility can reduce the chances of accidental exposures, however a more thorough cleaning of equipment and work surfaces is needed to eliminate the potential for accidental contamination.

The cleaning of laboratory equipment, work surfaces and work areas by decontamination, disinfection and sterilization methods is essential to eliminate or inhibit the ability of a contaminate microorganism from being spread throughout the work area or inoculating research material.

Selecting the most effective decontamination procedure or disinfectant will be dependent upon the physical limitations of the material being cleaned, how thorough the cleaning needs to be and the other potential contaminates that may be present.

Physical sterilization processes; i.e., heat and gas sterilization, are used on laboratory equipment and labware that are capable of withstanding the exposure to the process. Chemical disinfectants are used on those items or materials that are not designed to withstand heat or gas sterilization or may involve contact with living tissue.

By the general nature of the product, gas sterilants and chemical disinfectants are toxic and are to be handled according to the manufacturer's directions. Appropriate personnel protective equipment is to be worn when these materials are in use.

Definitions

Antiseptics: Chemical disinfectants that are designed to be used on living tissue surfaces. Antiseptics are less toxic than disinfectants used on inanimate objects. Due to the lower toxicity, antiseptics can be less active in the destruction of normal and any pathogenic flora present.

Autoclave: An apparatus used for sterilizing using superheated steam under high pressure. To sterilize using superheated steam under high pressure. (See Steam Sterilization)

Chemical Sterilant: A germicide that can destroy all forms of microbial life when adequate exposure conditions are realized. Chemical sterilants are often used as high-level disinfectants when shorter contact times are utilized.

Decontamination: The killing of organisms or removal of contamination after use, with no quantitative implication, generally referring to procedures for making items safe before disposal.

Disinfectant: A germicide that inactivates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms. May not be effective against bacterial spores.

Disinfection: The elimination or destruction of all pathogenic microorganisms. The term has been extensively misused and generally applies to the destruction of any pathogenic vegetative bacteria.

High-level: The elimination or destruction of all microorganisms with the exception of high numbers of bacterial spores.

Intermediate-level: The elimination or destruction of all vegetative bacteria including the *Mycobacteria*, most viruses, and most fungi but does not necessarily kill bacterial spores.

Low-level: The elimination or destruction of pathogenic vegetative bacteria, some viruses, and some fungi but not *Mycobacteria* or bacterial spores.

Germicide: An agent that destroys microorganisms, particularly pathogenic microorganisms.

Sanitization: The process of reducing microbial contamination to an acceptable "safe" level. The process of cleaning objects without necessarily going through sterilization.

Steam Sterilization: Autoclave, the process of sterilization by the use of heated steam under pressure to kill vegetative microorganisms and directly exposed spores. Common temperature and pressure for being effective is 121°C (250°F) at 15 psi (pounds per square inch) over pressure for 15 minutes. Special cases may require a variation of the steam temperature and pressure used.

Sterilization: The complete elimination or destruction of all forms of life by a chemical or physical means. An absolute not a relative term.

Sterilization

Steam Sterilization

The use of steam under pressure is perhaps the most efficient means of sterilization and is widely used in laboratory and medical facilities to sterilize equipment, glassware, and contaminated materials. All pathogenic bacteria, both vegetative and spore forms, are destroyed within twelve minutes of exposure and direct contact to pure steam heat of 121°C (121°F). Most are destroyed within seconds of exposure. Pure steam at a pressure of 15 psi (pounds per square inch), one atmosphere over pressure, corresponds to the temperature of 121°C. Adequate time must be permitted to attain the 121°C for an exposure of at least 12 minutes for all portions of the articles that are being steam autoclaved. Because of the necessity to allow for adequate exposure for all portions of the materials that are being autoclaved it is necessary to increase the minimum exposure time to 15 minutes. The duration of time needed to adequately heat sterilize material will be dependent upon the quantity and type of material being sterilized at one time, the larger the load the longer the time needed to achieve the needed temperatures deep within the load.

The effectiveness of a routine steam sterilizing cycle can be determined by using the appropriate biological indicator (**Appendix C**), ampoules or test strips containing *Bacillus stearothermophilus* spores or a spore enzyme (a-D-glucosidase) based rapid readout test. There are also several chemical indicators that can also provide reliable information. The standard biological indicator that is used in monitoring the effectiveness of steam sterilization are the *Bacillus stearothermophilus* spores because the spores are highly resistant to high temperatures. The use of the spore enzyme test is increasing in popularity because of its ability to provide results within 3 hours of exposure. The use of temperature sensitive autoclave tape can be misleading since the tape is only capable of indicating that a general temperature was reached. It does not indicate how long the material was exposed to the high temperature.

Autoclaved biological indicator samples should be examined for growth following an exposure to an actual autoclave cycle. The presence of growth in a *Bacillus stearothermophilus* sample or the presence of a color or of a fluorescing color change in other indicators after being steam autoclaved indicates that the exposure cycle was not adequate and must be repeated. In addition to the use of a biological indicator for determining the effectiveness of an autoclave cycle it is important that the researcher be aware of any special handling requirements that may be needed to effectively neutralize their cultured microbial agent or contaminated laboratory equipment. The researcher must understand and handle potentially infectious materials accordingly to reduce the potential for exposure.

The types of materials that may be steam sterilized in an autoclave can be varied in form; by shape and size, solid or liquid in composition or a combination of all, and the autoclave must be capable of accommodating for the type of load. The type of load to be autoclaved will determine the type of steam sterilizing cycle to be used; a liquid load requires a slow depressurization to prevent the liquid from boiling over once the autoclave pressure is reduced.

There are a number of different manufactures and different model designs of steam autoclaves. Before using any steam autoclave, the operation instructions for proper use and timing

requirements must be reviewed. Operators of a steam autoclave must remember that a steam autoclave is operated under pressure and at elevated steam temperatures. Failure to review the operational directions can result in improper sterilizing cycle being used, damage to the materials being exposed to the steam heat, damage to the autoclave and potentially serious or fatal injuries of the operator. Personal injuries can result from steam burns and from not allowing the autoclave to depressurize properly. If a steam autoclave is not working properly do not use the unit until it is repaired, contact the responsible person for the unit and inform them of the problem and label the unit "Out of Service".

In accordance with Wright State University policies dealing with the handling of infectious waste materials, infectious waste materials are to be disposed of according to university procedures defined in **Appendix D**, *Infectious Waste Management Guide* of this manual.

Not all materials are capable of being exposed to steam sterilization in an autoclave. For those items that can not be steam sterilized there are other alternatives in the form of gas sterilization or chemical disinfectants that can be used given proper consideration to practicality, the desired level of disinfection and potential hazards associated with handling of the item and the disinfectant.

Gas Sterilization

Ethylene oxide and formaldehyde gases are generally used for gas disinfection as fumigants under controlled conditions. Ethylene oxide and formaldehyde require special handling procedures to minimize potential personal exposure. Both materials are considered to be suspect carcinogens according to OSHA and an occupational carcinogens according to NIOSH.

Ethylene Oxide (CAS #75-21-8)

Ethylene Oxide (ETO) is used primarily as a means of sterilizing materials that are not designed to be exposed to steam sterilization. The use of ethylene oxide on sensitive plastics, medical and biological preparations and other heat sensitive equipment has contributed to revolutionizing developments in the medical field. Early testing found that ethylene oxide was very effective as a killing agent of bacteria, spores, molds and viruses.

Studies that were conducted to identify the method of activation involved in the destruction of exposed microorganisms found that ethylene oxide caused the replacement of a labile hydrogen with an alkyl group on hydroxyl, carboxyl, sulfhydryl, amino and phenolic groups. The alkylation of these compounds in organisms affects cellular function and structure which leads ultimately to inactivation of cellular function and ultimately death.

As effective as ethylene oxide is as a gas sterilizer, it has some major drawbacks that are potentially hazardous that limit its use in a general laboratory environment. Ethylene oxide is a highly flammable and potentially explosive gas. The gas has an explosive concentration range of 3 to 100 percent, and it is listed as a suspect human mutagen and carcinogen. Because of the potential health risks and flammability potentials there are special handling and ventilation requirements that must be used when handling ethylene oxide. Due to the hazards associated with potential exposures OSHA has listed an

exposure limit of 1 ppm for the duration of a work day. Ethylene oxide is a gas at room temperature and is not to be used in the open environment of the laboratory due to its volatility and health affects.

Ethylene oxide sterilizers are specifically designed to either use a mixture of ethylene oxide and carbon dioxide (10:90) or to use 100 percent ethylene oxide. Before an ethylene oxide sterilizer is to be used the unit should be checked for integrity and the operator must be familiar with operational procedures. The exposure time for a sterilization cycle is usually 4 to 6 hours in duration followed by a period of ventilation to allow for thorough dissipation of absorbed gas. The venting of the sterilizer following use is necessary, exposure to the residual material can be damaging to skin and may present a potential fire hazard.

To test for proper operation of an ethylene oxide sterilizer the biological indicator *Bacillus subtilis* var. niger is used. The spores from *B. subtilis* were found to be highly resistant to the effects of exposure to ethylene oxide

If ethylene oxide is being used in the laboratory it is the laboratory supervisor's responsibility to review all relevant safety information in the safe use, handling and disposal of this material and to be certain that others working in the laboratory receive appropriate training and warnings. Contact the Department of Environmental Health and Safety for assistance in assessing the potential for personal exposures and evaluation of laboratory handling procedures.

Formaldehyde (CAS#50-0-0)

Formaldehyde gas is most frequently used in the process of performing space fumigation of a room or of a piece of laboratory equipment that operated with a controlled environment. At the present time the only accepted method available for decontaminating a biological safety cabinet is to use formaldehyde gas. Formaldehyde gas for decontamination of a biological safety cabinet is generated by heating flaked or powdered paraformaldehyde in the presence of an elevated humidity of nearly 65 percent. Paraformaldehyde generates formaldehyde gas when it is depolymerized by heating to 232 to 246°C (450 to 475°F); the depolymerized material reacts with the moisture in the air to form formaldehyde gas. Using a balanced amount of ammonium bicarbonate neutralizes the formaldehyde gas within the biological safety cabinet. Only individuals that have specific training are permitted to decontaminate biological safety cabinets.

In areas where formaldehyde may be used for fumigation it is important to be aware of potential contacts with incompatible materials that could cause the formation of dangerous reaction products. Clear all materials out of an area where formaldehyde may be used to minimize the chance of a possible reaction with incompatible chemicals. Formaldehyde can react violently or explosively when exposed to incompatibles; in the presence of strong oxidizers there is a chance of fire and explosion or when exposed to hydrogen peroxide there is a violent reaction. Most notable however, formaldehyde may combine with hydrochloric acid or hydrogen chloride to form *bis*(chloromethyl) ether (BCME), a carcinogenic compound.

OSHA, NIOSH and IARC recognize formaldehyde as a suspect carcinogen. OSHA has established an exposure limit of 0.75 ppm during a workday. The Department of Environmental Health and Safety can

evaluate work tasks and perform monitoring tests to determine the potential for an occupational exposure.

Chemical Disinfectants

Choosing a Chemical Disinfectant

A variety of concerns must be addressed when choosing a disinfectant for use in a biohazard area. No one disinfectant is universally ideal and the decision as to the optimum disinfectant involves the consideration of factors such as:

Organism susceptibility
Material or surface to be disinfected
Organic load of the material being disinfected
Potential health risks to laboratory personnel
Hazardous properties of the disinfectant (i.e., flammable, corrosive, toxic)
Stability of the disinfectant
pH, temperature and presence of other contaminants in media and water for dilution
Required contact time for effective disinfection
Requirements for disposal of the disinfectant
Cost

Choosing a disinfectant is, therefore, a decision that requires a fairly detailed knowledge of the target organism, a basic knowledge of disinfectants, and careful consideration of the above factors as they apply to the unique potential conditions in which your laboratory will employ the disinfectant. Always consult the product information, the material safety data sheet (MSDS), on a disinfectant before using the material. Appropriate personnel protective equipment is required to be worn when materials are being mixed and used.

For the chosen chemical disinfectant to be affective when used it must be able to make direct contact with the target organism. Environmental factors such as air bubbles, grease, dirt, a dense concentration of microorganisms and the presence of other chemicals (i.e., soaps) can reduce the effectiveness of the disinfectant.

The Halogens

Chlorine

Chlorine is one of the least expensive and most effective disinfectants. The recommended concentration of sodium hypochlorite for "clean surface" disinfection is 200 ppm, representing approximately a 1:250 dilution of household bleach. The CDC recommends a 1:10 dilution of household bleach as the disinfectant of choice for blood spills while many laboratory safety texts recommend the use of undiluted household bleach for biohazard spill containment. These varying recommendations occur primarily because of chlorine's easy inactivation by organic material (serum, blood, proteins, etc.) and the fact that chlorine's disinfectant activity, unlike many of the other disinfectants, increases as the concentration increases.

Of all the disinfectants, chlorine has one of the most extensive ranges of organisms that are susceptibility to destruction under ideal circumstances. All of the vegetative bacteria that have been tested are susceptible to chlorine destruction, including the acid-fast bacteria. Bacterial spores are also susceptible although longer exposure times are generally required. Both enveloped and non-enveloped viruses are susceptible to chlorine inactivation.

One of the main disadvantages of chlorine as a disinfectant is the ease with which it is inactivated by organic material. Materials to be disinfected should be first cleaned to remove the organic material or the concentration of the chlorine must be increased to compensate for the organic material inactivation. Chlorine is also easily inactivated by a variety of metals including copper, zinc, nickel, iron, etc. and the use of chlorine as a disinfectant on these materials requires increased concentrations of chlorine, often resulting in damage to the substrate materials being disinfected.

Chlorine disinfectant solutions are also extremely sensitive to pH and the sensitivity has dramatic implications on the effectiveness of these solutions. Chlorine solutions are most active under slightly acid conditions (pH 6 to pH 7), the activity level decreases rapidly under conditions where the pH goes from a pH 7 to pH 8.5. As the pH of chlorine solutions increases the disinfectant activity levels decreases.

The limited pH range in which chlorine is effective, slightly acid to slightly basic, is also a limiting factor necessitating the use of nonionic detergents or precleaning followed by thorough rinsing.

A number of alternative forms of chlorine exist to use in the form of household bleach (sodium hypochlorite). Chlorine dioxide compounds are high level disinfectants/steriliants that offer somewhat increased activity and resistance to organic inactivation in comparison to household bleach. Chloramine-T and other organic chlorine compounds also offer increased resistance to organic inactivation but at the cost of decreased activity. While these compounds offer specific advantages, household bleach remains one of the best disinfectants available.

Important Information when Considering to Use Hypochlorite Solutions:
Three situations exist where the uses of hypochlorite solutions pose a potential risk to personnel using the compound. First, the addition of acid to hypochlorite solutions will produce a rapid production of toxic chlorine gas. Second, the contact of chlorine solutions with formaldehyde produces the carcinogen bis-chloromethyl ether. Lastly, the heating of chlorine solutions produces the carcinogen trihalomethane. Chlorine solutions, therefore, must never be autoclaved.

Iodine
Iodine-based disinfectants share the same properties as the chlorine-based disinfectants but are somewhat less reactive with substrates and microorganisms. Like chlorine disinfectants, the iodines are effective against vegetative bacteria, acid-fast bacteria, bacterial spores, and both enveloped and non-enveloped viruses although longer contact times are generally required under similar conditions. Most of the iodine-based disinfectants utilized in laboratory and medical situations are combinations of elemental iodine or triiodide with a neutral polymer carrier molecule. These compounds are collectively referred to as iodophors. Iodophors are excellent disinfectants and antiseptics and are extensively used for surgical scrub solutions, hand-washing compounds, and disinfectants for small laboratory objects.

Unlike the elemental chlorine and iodine, however, iodophors are extremely sensitive to concentration and are quite expensive.

Alcohols

Ethyl and Isopropyl Alcohol

Ethanol and Isopropyl alcohol are both excellent disinfectants whose germicidal properties are generally underestimated. Both are rapidly bacteriocidal against vegetative bacterial forms, tuberculocidal, fungicidal, and virucidal. Neither inactivates bacterial spores and isopropyl alcohol fails to inactivate hydrophilic viruses. Both ethanol and isopropyl alcohol should be considered as intermediate-level disinfectants.

One of the most critical factors in the use of alcohols as disinfectants is concentration. The disinfectant properties of both ethanol and isopropyl alcohol rapidly drop at concentrations below fifty percent (50%) and above ninety percent (90%). Peak disinfectant activity occurs at approximately sixty-seven percent (67%) concentration. The recommended concentration for use is sixty - ninety percent (60 - 90%) by volume.

Both ethanol and isopropyl alcohol are volatile and flammable compounds and must only be used with adequate ventilation. Alcohols, in general, are destructive to rubber compounds and to most of the cement and glues used in instruments, especially optics.

Phenolic Compounds

Phenol

Ever since the adoption of carbolic acid by Lister as the first germicide, phenols have been extensively used. Numerous studies, beginning with a study by Kronig and Paul in 1897, have explored the various chemical substitutions and their effect upon germicidal properties. Today, the only phenolic derivatives found in extensive use, as disinfectants are *o*-phenylphenol, *o*-benzyl-*p*-chlorophenol, and *p*-tert-amylphenol. The mode of action of phenolic compounds appears to be a generalized cytoplasmic poisoning at higher concentrations and an inactivation of enzyme systems and cell wall integrity at lower concentrations.

Overall the phenolic derivatives are all characterized by a broad-spectrum of activity against gram-positive and gram-negative bacteria, fungicidal, tuberculocidal, and virucidal activity against lipophilic viruses (enveloped viruses). Phenols have a high tolerance to both organic load and hard water. Their use also results a residual activity on surfaces. Overall, phenolic derivatives are best classified as low- to intermediate- level disinfectants appropriate for general use in noncritical or semicritical areas. They lack sporicidal activity and are ineffective against noneveloped viruses. Phenol should never be used for sterilization purposes.

Phenolic compounds may exhibit dramatic toxic effects. Phenol compounds rapidly penetrate porous compounds and tend to accumulate in the body fat of exposed animals. Reports of phenolic disinfectant induced skin depigmentation, nerve demyelination and skin contact dermatitis that requires personnel using phenolic disinfectant be provided with appropriate protective clothing and equipment.

Two halogenated phenolic derivatives; parachlorometaxylenol (PCMX) and 2,4,4'-trichloro-2-hydroxydiphenol (Triclosan, Irgasan), are commonly used as antibacterial agents in soaps and scrubs as well as preservatives in a number of products. PCMX has become the most widely used antiseptic scrub in surgery and is used as a preservative in products ranging from printing inks to cosmetics to shoe polishes. Triclosan is now commonly used in antibacterial soaps and deodorants as well as being incorporated into plastics as a "permanent" (but questionable) antibacterial.

Chlorhexidine

Discovered during a search for potential anti-malarial drugs, chlorhexidine proved to have a high level of antibacterial activity, low mammalian toxicity, and a strong affinity for binding to skin and mucous membranes, all of which are desirable characteristics for an antiseptic. Chlorhexidine compounds are generally active against gram-positive and gram-negative vegetative bacteria and lipophilic viruses. Many fungi are sensitive to chlorhexidine and acid-fast bacteria are generally inhibited but not killed (bacteriostatic). Bacterial spores are not killed but germination is inhibited while in contact with chlorhexidine.
Chlorhexidine's activity at relatively low concentrations involves a series of related cytologic and physiologic changes culminating in ion leakage from the cytoplasmic membrane and cytoplasmic precipitation. Chlorhexidine's primary advantage over other disinfectants and antiseptic agents involves both its rapid rate of bacteriocidal activity and its strong binding to skin and mucous membranes.

Chlorhexidine is best classified as a low- to intermediate- level disinfectant appropriate for noncritical and semicritical area disinfectant. As an antiseptic, the lack of direct tissue toxicity and the rapidity of action makes chlorhexidine an excellent bacteriocidal skin cleanser and wound cleaning agent.

Quaternary Ammonium Compounds

Quaternary ammonium disinfectants (quats) first appeared in the late 1930's. Since the original introduction, there has been the addition of numerous compounds, blends, different adjunctive agents, etc., making the entire group of quaternary ammonium disinfectants a rather broad group with a variety of activities, advantages, and disadvantages. The major advantages that are common to the group are an inherent surfactant activity, allowing them to also serve as cleansing agents, and a relatively low level of mammalian toxicity. Common disadvantages include a lack of sporicidal activity and a lack of activity against acid-fast bacteria (except for some of the latest generation quats).

The first generation of quaternary ammonium compounds were the standard benzalkonium chloride compounds developed in the 1930's. Substitution of the aromatic ring hydrogen with chlorine, methyl, and ethyl groups resulted in increased activity and the generation of the second generation of quaternary ammonium compounds. The third generation of quaternary ammonium compounds, or the dual quats, were developed in 1955 and represented compounds with superior microbiological activity. Presently, the quaternary ammonium compounds, now polymeric and polysubstituted quaternary ammonium compounds, are in the seventh generation of development. The newest generation of quats possess a wide spectrum of activity with minimal mammalian host damage and are used in pharmaceuticals, ophthalmic solutions, and contact lens solutions, etc.

The antimicrobial activity of quaternary ammonium compounds appears to be by inactivation of critical enzyme systems. Inactivating substances vary dramatically between the generations of quats with the later generations generally much less susceptible to inactivation by extraneous material such as organic load or hard water.

As far as choosing a quaternary ammonium disinfectant, it is critical to read the label directions on the bottle. Organism susceptibilities differ dramatically between different generations of quats and different formulations.

Appendix F
Internet and Available Publications for Biosafety Resources

With access to the world wide web, the internet can be a valuable asset to the researcher or the laboratory associate searching for additional information on a topic of interest or seeking general information. With a few starting points there are a multitude of locations that can be accessed to provide information as well as names of persons to contact. If you have access to the internet here are some locations that can a valuable resource location.

In addition to the resources on the internet there are publications available for review through the university libraries and the Department of Environmental Health and Safety. If there are additional publications that would be of benefit to others and are not listed please forward the information to the Department of Environmental Health and Safety for consideration.

I. Internet Access: (URL's)

A. Academic Institutions:

Duke University, Occupational and Environmental Safety Services (OESO)
www.safety.duke.edu/department/index.html

Florida State University, Environmental Health & Safety
www.fsu.edu/~safety/bio.htm

Michigan Sate University, Office of Radiation, Chemical and Biological Safety (ORCBS)
www.msu.edu

(The) Ohio State University, Office of Environmental Health and Safety, BioSafety Program
www.ehs.ohio-state.edu/biosafety.html

Oklahoma State University, Environmental Health & Safety
www.pp.okstate.edu/ehs

Stanford University Department of Environmental Health and Safety
www.-leland.stanford.edu/dept/EHS/index.html

State University of New York at Buffalo, Occupational and Environmental Services (OES)
http://wings.buffalo.edu/services/fac/oes

University of California San Diego, Environmental Health and Safety
http://www-ehs.ucsd.edu

University of California Santa Barbara Department of Environmental Health and Safety
http://ehs.ucsb.edu/programs/f-programs.html

University of Florida, Environmental Health and Safety, Biological Safety Program
www.ehs.ufl.edu/bio/default.asp

University of Wyoming, Environmental Health and Safety
http://safety.uwyo.edu/ehs

Wayne State University, Office of Environmental Health & Safety
www.science.wayne.edu/~oehs

Yale University, Office of Environmental Health & Safety
www.yale.edu/oehs/ohsp.htm

B. Agencies and Organizations:

Agency for Toxic Substances and Disease Registry (ATSDR)
http://atsdr1.atsdr.cdc.gov.8080/toxfaq.html

American Biological Safety Association (ABSA)
www.absa.org

Biosafety Resources - directs internet access to:
X Risk Group Classification for Infectious Agents (print table or as PDF file)
X MSDS's for Infectious Agents
X Rules, Regulations and Permits (Permits, rDNA, Infectious Agent Rules)
X Requirements for Facilities Transferring or Receiving Selected Agents
X Agencies and Organizations (ABSA, ASM, ATSDR, CDC, FDA, NSF, etc.)
X Biosafety Pages from other Academic Institutions and other organizations
X Laboratory Biosafety Guidelines (*Biosafety in Microbiological and Biomedical Laboratories*, *Use of Biological Safety Cabinets*, *Guidelines for Transport of Infectious Substances and Diagnostic Specimens*, *NIH Guidelines for rDNA*, and *LCDC Canada, Lab Biosafety Guidelines*)
X Online Journals (MMWR, Medscape, Texas A&M Medical Sciences Library)
X Products and Services (available Commercial Training Courses, Certifications and Equipment)
-Guest Book - How to participate in the Biosafety Discussion Listserv (BIOSAFTY)
-What's New
- Information postings from the ABSA (job postings, articles of information, reviews)

Center for Disease Control (CDC)
www.cdc.gov

Extoxnet - Information on Pesticides
http://ace.orst.edu/info/extoxnet

Laboratory Centra for Disease Control - Canada, Office of Biosafety
www.hc-sc.gc.ca/main/lcdc/web/biosafty/index.htm

National Institute of Health (NIH)
www.nih.gov

National Sanitation Foundation - Biological Safety Cabinets
A Listing of Certified Equipment
www.nsf.org/certified/Cabinets.cfm

NSF Field Service Technicians
www.nsf.org/certified/Cabinets.cfm#Accredit

NIOSH (National Institute of Occupational Safety and Health)
www.cdc.gov/niosh

OSHA (Occupational Safety and Health Administration)
www.osha.gov

U.S. Environmental Protection Agency (USEPA)
www.epa.gov

C. Biological Safety Cabinets - Manufacturers with NSF certified cabinets

Baker Co.	www.Bakerco.com/hiband
FormaScientific	www.Forma.com/BCpg1.html
Heraeus*	www.heraeus-instruments.de/home.cfm?=E&uid=55490

*this company is new to the US market, NSF certification is pending

Labconco	www.Labconco.com
NuAire	www.NuAire.com

D. Institutional Biosafety Committees Home Page (Contains site locations for Institutional Biosafety Committees at other institutions)
www.nbiap.vt.edu/ibc-url.html

E. Publications, available on the Internet

Biological Material Safety Data Sheets (MSDS=s) from Health Canada, Health Protection Branch, Laboratory Centre for Disease Control

www.hc-sc.gc.ca/hpb/lcdc/biosafty/msds/index.html

Biosafety in Microbiological and Biomedical Laboratories, 4th (CDC/NIH)

www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Guidelines for Use of Biosafety Cabinets

www.cdc.gov/od/ohs/biosafty/bsc/bsc.htm

Mortality and Morbidity Weekly Report (MMWR) (CDC)

www.cdc.gov/epo/mmwr/mmwr.htm

Office of BiotechnologyActivities (Formerly called: Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

www.nih.gov/od/oba/

F. Search Engines on the Web

Use of search engines such as AOL.COM, HotBot, Lycos, Netscape and Yahoo and by using the key word *biosafety* to obtain hits on locations found within the world wide web. The subsequent search of hits can lead to a variety of sources of information for review.

II. Publications Available for Reference:

Block, Seymore S. (editor), 1991, **Disinfection, Sterilization and Preservation**, 4th ed., Lea & Febiger (Fordham HSL Library) (Call No. QV220 D611 1991)

Fleming, Diane O.; Richardson, John; Tulis, Jerry J.; and Vesley, Donald (editors), 1995, **Laboratory Safety, Principles and Practices**, Second Edition, ASM Press (EHS Office)

Liberman, Daniel and Gordon, Judith G., 1989, **Biohazards Management Handbook**, Marcel Dekkar Inc. (EHS Office)

Journal of the American Biological Safety Association, American Biological Safety Association National Research Council, 1989, **Biosafety In The Laboratory, Prudent Practices for the Handling and Disposal of Infectious Materials**, National Academy (EHS Office)

NSF Listings: Biohazard Cabinetry, Class II Cabinet Certification and Field Certifier Accreditation, July 1995 - current issue

Rayburn, Stephen R., 1990, **The Foundations of Laboratory Safety, A Guide for the Biomedical Laboratory**, Springer-Verlag (EHS Office)

Richmond, Jonathon, editor, 1999, **Anthology of Biosafety, I. Perspectives of Laboratory Design**, American biological Safety Association (EHS Office)

Richmond, Jonathon Y. and McKinney, Robert W., 1999, **Biosafety in Microbiology and Biomedical Laboratories**, 4th edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institute of Health (EHS Office)

Richmond, Jonathon Y. and McKinney, Robert W., 1993, **Biosafety in Microbiology and Biomedical Laboratories**, 3rd edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institute of Health (EHS Office)

Richmond, Jonathon Y., Ph.D. (editor), 1997, **Designing a Modern Microbiological/Biomedical Laboratory, Lab Design Process and Technology**, American Public Health Association (EHS Office)

Ryan, Kenneth J., (editor), 1994, ***Sherris*** **Medical Microbiology, An Introduction to Infectious Diseases**, 3rd edition, Appleton &Lange (EHS Office)

Appendix G
Selection and Proper Use of Biological Safety Cabinets

New Installation Planning:

- Identify the proper type of biological cabinet that is needed for the work that is going to be performed. Consult with the Department of Environmental Health and Safety for assistance in selecting the right cabinet.
- The placement of the biological safety cabinet in the work area is important. Do not place the cabinet near an exit door, in an area of heavy traffic or under air supply ducts for the room. Activities that cause a disruption of the general airflow will affect the ability of the cabinet to perform properly.

Operational Limitations of Biological Safety Cabinets:

- Biological safety cabinets are not to be used as storage locations of equipment.
- A Type II Class A biological safety cabinet is not to be used with volatile chemicals. The Class A cabinet vents directly back into the room.
- For proper performance and to provide the protection that the cabinet is designed to provide the cabinet must be maintained and serviced as necessary. Failure to maintain the cabinet can result is situations where either, or both, product or personal protection is compromised.

General Procedures:

- Do not use the top of a cabinet for storage. The exhaust filter can be easily damaged by materials falling onto the exhaust filter.
- Do not use the cabinet for long term storage of materials. Keep the inner work surface clear of materials between procedures.
- The biological cabinet is to be field certified at least on an annual basis. The cabinet will meet the NSF Standard 49 (Class II Biohazard Cabinetry) or the manufacturer's specifications for proper operation.
- Identify the chemicals that are going to be used and obtain a Material Safety Data Sheet (MSDS) for those items. Review the information contained in the MSDS with personnel before the material is put into use. The MSDS must be readily available for review.
- Identify any special precautions associated with biological agents that are going to be manipulated. Have written procedures for special handling and cautions.
- Identify and establish written procedures for the use of the biological safety cabinet and for spill containment.
- Maintain a use log of organisms or agents used in the cabinet. The log will also contain a record of all maintenance performed. The log will be a permanent record for the cabinet.

Preparation for Use:

- If a UV light is present in the cabinet, the UV light is to be turned OFF whenever people are going be working in or in the area of the cabinet.
- Check the drain valve to make sure that it is closed. To be closed the valve handle should be parallel to the bottom of the cabinet (at a right angle to the valve).
- If the cabinet is OFF, turn ON the blower to initiate the airflow within the cabinet.
- If the front sash is movable, set the sash at the proper operational height for the cabinet.
- The work surface should be disinfected with an appropriate disinfectant that will kill the microorganisms that are likely to be present.
- Allow the cabinet to operate for 5 minutes to establish air flow patterns.
- Check the cabinet for proper operation; ie., check the magnehelic gauge for reference.

Note: *If the cabinet is NOT operating properly, as designed, DO NOT use the cabinet until the problem has been corrected. Failure to have an operational problem corrected can result in the exposure of the operator to the agents that are being manipulated in the cabinet with the potential of serious consequences.*

- Place only needed equipment inside the cabinet, do not block or obstruct the front or rear ventilation grilles of the cabinet.
- Establish work zones within the work surface area to minimize cross contamination, clean materials should be upstream of contaminated areas.
- Do not place upright pipette collectors in the cabinet use a flat collection tray with a cover to contain used pipettes.
- A biohazardous material collection bag should be placed inside the cabinet to contain biohazardous waste material. Passing generated biohazardous waste material outside of the cabinet to a collection bag may cause the operator to be exposed to aerosolized infectious materials.

Working in the Cabinet:

- Wash hands before starting to work in the cabinet.
- The use of PPE (personal protective equipment) is required even while working in a biological safety cabinet. ANSI approved safety glasses or goggles are to be worn, a lab coat with appropriate gloves. Wearing of shorts and sandals are discouraged while working.
- No eating, drinking or chewing of gum while working in a biological safety cabinet.
- Only one person is to work in a cabinet at a time.
- Do not use volatile organic compounds in a Class II Type A biological safety cabinet.
- Adjust seating height so that the operator's arms do not rest on the front edge or block the front grille.
- Do not use open flames in a biological safety cabinet. If a flame source is needed use a touch-o-matic type flame source.

- Movements in and out of the cabinet while working should be straight in and out and should be kept to a minimum, avoid sweeping movements that will cause a disruption of the airflow profile within the cabinet.
- Use the biohazardous material collection bag inside of the cabinet to contain biohazardous waste and to reduce to passing of the operator's arms through the clean zone of operation.

Post Procedure Clean Up:

- Potentially contaminated equipment within the cabinet must be surface disinfected before being removed from the cabinet.
- Use an appropriate disinfectant on the work surface once cleared of equipment.
- After all potentially contaminated equipment has been disinfected, remove gloves and wash hands.
- If the cabinet blower is going to be turned OFF, allow the cabinet to run for several minutes to perform a final purge of the cabinet.
- If a UV light is activated, pull the front sash down to close off the front opening of the cabinet. DO NOT activate a UV light if the sash is fixed in place and there are going to be people working in the vicinity of the cabinet.
- Do not store materials or supplies inside of a biological safety cabinet.

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- The placement of the biological safety cabinet in the work area is important. Do not place the cabinet near an exit door, in an area of heavy traffic or under air supply ducts for the room. Activities that cause a disruption of the general airflow will affect the ability of the cabinet to perform properly.

Operational Limitations of Biological Safety Cabinets:

- Biological safety cabinets are not to be used as storage locations of equipment.
- A Type II Class A biological safety cabinet is not to be used with volatile chemicals. The Class A cabinet vents directly back into the room.
- For proper performance and to provide the protection that the cabinet is designed to provide the cabinet must be maintained and serviced as necessary. Failure to maintain the cabinet can result is situations where either, or both, product or personal protection is compromised.

General Procedures:

- Do not use the top of a cabinet for storage. The exhaust filter can be easily damaged by materials falling onto the exhaust filter.
- Do not use the cabinet for long term storage of materials. Keep the inner work surface clear of materials between procedures.
- The biological cabinet is to be field certified at least on an annual basis. The cabinet will meet the NSF Standard 49 (Class II Biohazard Cabinetry) or the manufacturer's specifications for proper operation.
- Identify the chemicals that are going to be used and obtain a Material Safety Data Sheet (MSDS) for those items. Review the information contained in the MSDS with personnel before the material is put into use. The MSDS must be readily available for review.
- Identify any special precautions associated with biological agents that are going to be manipulated. Have written procedures for special handling and cautions.
- Identify and establish written procedures for the use of the biological safety cabinet and for spill containment.
- Maintain a use log of organisms or agents used in the cabinet. The log will also contain a record of all maintenance performed. The log will be a permanent record for the cabinet.

Preparation for Use:

- If a UV light is present in the cabinet, the UV light is to be turned OFF whenever people are going be working in or in the area of the cabinet.
- Check the drain valve to make sure that it is closed. To be closed the valve handle should be parallel to the bottom of the cabinet (at a right angle to the valve).
- If the cabinet is OFF, turn ON the blower to initiate the airflow within the cabinet.
- If the front sash is movable, set the sash at the proper operational height for the cabinet.
- The work surface should be disinfected with an appropriate disinfectant that will kill the microorganisms that are likely to be present.
- Allow the cabinet to operate for 5 minutes to establish air flow patterns.
- Check the cabinet for proper operation; ie., check the magnehelic gauge for reference.

Note: *If the cabinet is NOT operating properly, as designed, DO NOT use the cabinet until the problem has been corrected. Failure to have an operational problem corrected can result in the exposure of the operator to the agents that are being manipulated in the cabinet with the potential of serious consequences.*

- Place only needed equipment inside the cabinet, do not block or obstruct the front or rear ventilation grilles of the cabinet.
- Establish work zones within the work surface area to minimize cross contamination, clean materials should be upstream of contaminated areas.
- Do not place upright pipette collectors in the cabinet use a flat collection tray with a cover to contain used pipettes.
- A biohazardous material collection bag should be placed inside the cabinet to contain biohazardous waste material. Passing generated biohazardous waste material outside of the cabinet to a collection bag may cause the operator to be exposed to aerosolized infectious materials.

Working in the Cabinet:

- Wash hands before starting to work in the cabinet.
- The use of PPE (personal protective equipment) is required even while working in a biological safety cabinet. ANSI approved safety glasses or goggles are to be worn, a lab coat with appropriate gloves. Wearing of shorts and sandals are discouraged while working.
- No eating, drinking or chewing of gum while working in a biological safety cabinet.
- Only one person is to work in a cabinet at a time.
- Do not use volatile organic compounds in a Class II Type A biological safety cabinet.
- Adjust seating height so that the operator's arms do not rest on the front edge or block the front grille.
- Do not use open flames in a biological safety cabinet. If a flame source is needed use a touch-o-matic type flame source.

- Movements in and out of the cabinet while working should be straight in and out and should be kept to a minimum, avoid sweeping movements that will cause a disruption of the airflow profile within the cabinet.
- Use the biohazardous material collection bag inside of the cabinet to contain biohazardous waste and to reduce to passing of the operator's arms through the clean zone of operation.

Post Procedure Clean Up:

- Potentially contaminated equipment within the cabinet must be surface disinfected before being removed from the cabinet.
- Use an appropriate disinfectant on the work surface once cleared of equipment.
- After all potentially contaminated equipment has been disinfected, remove gloves and wash hands.
- If the cabinet blower is going to be turned OFF, allow the cabinet to run for several minutes to perform a final purge of the cabinet.
- If a UV light is activated, pull the front sash down to close off the front opening of the cabinet. DO NOT activate a UV light if the sash is fixed in place and there are going to be people working in the vicinity of the cabinet.
- Do not store materials or supplies inside of a biological safety cabinet.

APPENDIX I
OHIO ENVIRONMENTAL PROTECTION AGENCY
DEFINITIONS OF INFECTIOUS WASTE

A. "Infectious Agents" means a type of microorganism, helminth, or virus that causes, or significantly contributes to the cause of increased morbidity or mortality of human beings.

B. "Zoonotic Agent" means a type of microorganism, helminth, or virus that causes disease in vertebrate animals and that is transmissible to human beings and causes or significantly contributes to the cause of increased morbidity or mortality of human beings.

C. "Infectious Wastes" includes all of the following substances or categories of substances:

1. Cultures and stocks of infectious agents and associated biologicals, including, without limitation, specimen cultures, cultures and stocks of infectious agents, wastes from production of biologicals, and discarded live and attenuated vaccines;

2. Laboratory wastes that were, or are likely to have been, in contact with infectious agents that may present a substantial threat to public health if improperly managed;

3. Pathological wastes, including, without limitation, human and animal tissues, organs, and body parts, and body fluids and excreta that are contaminated with or are likely to be contaminated with infectious agents, removed or obtained during surgery or autopsy or for diagnostic evaluation, provided that, with regard to pathological wastes from animals, the animals have or are likely to have been exposed to a zoonotic or infectious agent

4. Waste materials from the rooms of humans, or the enclosures of animals, that have been isolated because of diagnosed communicable disease that are likely to transmit infectious agents. Also included are waste materials from rooms of patients who have been placed on blood and body fluid precautions under the universal precaution system established by the "Center for Disease Control" in the Public Health Service of the United States Department of Health and Human Services, if specific wastes generated under the universal precautions system have been identified as infectious wastes by rules referred to in section C.8. of this appendix;

5. Human and animal blood specimens and blood products that are being disposed of, provided that with regard to blood specimens and blood products from animals, the animals were or are likely to have been exposed to a zoonotic or infectious agent. "Blood products" does not include patient care waste such as bandages or disposable gowns that are lightly soiled with blood or other body fluids, unless such wastes are soiled to the extent that the generator of the waste determines that they should be managed as infectious wastes;

6. Contaminated carcasses, body parts, and bedding of animals that were intentionally exposed to infectious agents from zoonotic or human diseases during research, production of biologicals, or testing of pharmaceuticals, and carcasses and bedding of animals otherwise infected by zoonotic or infectious agents that may present a substantial threat to public health if improperly managed;

7. Sharp wastes used in the treatment, diagnosis, or inoculation of human beings or animals or that have, or are likely to have, come in contact with infectious agents in medical, research, or industrial laboratories, including, without limitation, hypodermic needles and syringes, scalpel blades, and glass articles that have been broken. Such wastes are hereinafter in this rule referred to as "sharp infectious waste" or "sharps";

8. Any other waste materials generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, that the Public Health Council created in Section 3701.33 of the Ohio Revised Code (ORC), by rules adopted in accordance with Chapter 119 of the ORC, identifies as infectious wastes after determining that the wastes present a substantial threat to human health when improperly managed because they are contaminated with, or are likely to be contaminated with, infectious agents;

9. Any other waste materials the generator designates as infectious wastes.

APPENDIX II
WRIGHT STATE UNIVERSITY
INFECTIOUS WASTE CONTAINMENT AND CLEANUP PROCEDURES

A. PRIMARY CONTACTS FOR INFECTIOUS WASTE SPILLS:

INSTITUTIONAL BIOSAFETY OFFICER:

Kim Morris	104 HS	775-2623

INFECTIOUS WASTE CONTROL MANAGER:

Bill Palmer	047 BS	775-3788
ENV. HEALTH AND SAFETY DEPT:	047 BS	775-2215
CAMPUS POLICE:	060 Allyn Hall	775-2111

B. LOCATION OF SPILL CONTAINMENT AND CLEANUP MATERIALS/KITS:

- Mathematics and Microbiology Building, hallway, Rooms 016 & 012
- Biological Sciences, Phase I, Room 117.
- Environmental Health and Safety, Room 141B Biological Sciences, Cabinet #24
- Student Health Services, 051T Student Union
- Disability Services, B072 Student Union
- Nutter Center, Athletic Training Area, Room 140
- Department of Human Biology, Room 150B, WSU Research Park
- Infectious Waste Storage Bldg., Parking Lot #20
- Cox Heart Institute, Room 223
- Diggs Laboratory, Outside Room 104A
- "grab-n-go" kit, 137 Biological Sciences
- Health Sciences, 232, under sink
- EHS Truck (rear), Diggs Lot

C. INFECTIOUS WASTE SPILL CONTAINMENT AND CLEANUP PROCEDURES:

In the event of an accidental spill or release of infectious waste at Wright State University or at a Wright State University affiliated facility, the following procedures must be implemented by the personnel responsible for the spill.

1. Immediately following the spill/release, secure the area so only authorized personnel may enter. Remove and containerize any contaminated garments immediately. Contact one of the primary contacts on the first page of this plan. At that time a determination will be made as to what, if any, outside agencies or support groups shall be contacted. If the spill/release occurs after 5:00 p.m. or on a Saturday, Sunday or holiday, contact Public Safety, ext. 2111. Public Safety will contact appropriate Environmental Health and Safety personnel.

2. Go to the nearest spill containment and cleanup kit and obtain all necessary containment, cleanup and protective equipment needed to effectively handle the spill/release involved. The location of the spill containment and cleanup kits are located on the first page of this plan.

3. Further secure the area to authorized personnel if needed.

4. Each person involved in the cleanup shall wear protective equipment as needed. This equipment is to be worn during the entire spill cleanup operation.

5. Wait 30 minutes before reentering the spill area to allow for settling of aerosols.

6. Spray spill area with disinfectant spray from the spill containment and cleanup kit or use a bleach solution (minimum 15% bleach to water) if the spill is large. Begin at the perimeter of the spill and work inward. Allow the disinfectant to remain in contact with the spilled material for at least twenty minutes before proceeding.

7. Use the absorbent pads supplied from the spill containment and cleanup kit to absorb any freestanding liquid.

8. Place all used absorbent pads and other waste generated during the cleanup into a biohazard bag supplied from the spill containment and cleanup kit. Absolutely no freestanding liquid or containers of liquid shall be put in any biohazard bag. All freestanding liquid shall be absorbed prior to placing it in the biohazard bag.

9. Use the chemical disinfectant supplied from the spill containment and cleanup kit to disinfect the entire area again. Clean up the area as deemed appropriate (i.e., use of absorbent pads). Any absorbent pads used during the disinfection of the area shall be considered infectious waste and placed in the biohazard bags.

10. Clean and disinfect all non-disposable items using the disinfectant supplied from the spill containment and cleanup kit.

11. Remove protective equipment and manage disposable items as infectious waste (place in biohazard bags).

12. Immediately following spill/release cleanup, all infectious waste generated from cleanup operations must be stored and managed as specified in Wright State University's "Infectious Waste Management Guide."

13. Notify Environmental Health and Safety, ext 2215, if material from a spill containment and cleanup kit was used to respond to the spill.

APPENDIX III
WRIGHT STATE UNIVERSITY SHARPS MANAGEMENT POLICY

As a university that generates infectious waste, individuals on campus must be aware of recent interpretations made by the EPA during inspections at WSU regarding the management of sharps. The following procedures must be followed as interpreted by the EPA per the requirements of Ohio Administrative Code 3745-27-34(B).

The EPA considers needles, razor/scalpel blades, lancets, broken glassware, pipettes, pipette tips, and syringes (even without needles) to be sharps. All of these sharps must be accumulated for disposal immediately into an approved sharps container. The type of container required depends on the type of sharps and whether or not the sharps are considered infectious. The EPA separates sharps into two categories. These are:

Maximum puncture potential sharps – These included needles, razor/scalpel blades, lancets, and any other sharp deemed to pose a definite puncture potential.

Minimum puncture potential sharps – These include pipettes, pipette tips, syringes without needles, and large broken glassware

Identify the type of sharps you generate and determine if they are infectious. Refer to Appendix I of the Infectious Waste Management Guide to review the definition of infectious waste. Once you have identified the type of sharp you generate and determined its infectious nature refer to the category below and manage your sharps accordingly.

Maximum Puncture Potential Sharps that **are** Infectious:

An approved sharps container is a puncture and leak resistant container specifically designed and manufactured for the accumulation of sharps and labeled with the words "Infectious Waste" or with the international biohazard symbol (i.e. a red sharps container). This type of sharps container, when full, shall be put in an infectious waste box supplied by EHS that also must marked as containing sharps and will be managed by EHS.

Maximum Puncture Potential Sharps that **are not** Infectious:

An approved sharps container is a puncture and leak resistant container that can be specifically designed and manufactured for the accumulation of sharps or could be a can or plastic container with a secure fitting lid that can be closed when full. In any case, these containers **must not** be labeled with the biohazard label or any other marking that would indicate it is infectious. The container must be marked with the word "SHARPS and when full can be put into a non-contaminated broken glass box provided by Environmental Services (formally Custodial Services). You must then mark the box as containing "SHARPS". The box, when full, can be managed as regular solid waste by Environmental Services personnel.

Minimum Puncture Potential Sharps that **are** Infectious:

An approved sharps container is an infectious waste box supplied by EHS. These types of sharps must be accumulated immediately into the box after use. The infectious waste box must then be marked as containing sharps and will be managed by EHS.

Minimum Puncture Potential Sharps that **are not** Infectious:

An approved sharps container is a broken glass box provided by Environmental Services. This box must be labeled as "SHARPS". This box, when full, can be managed as regular solid waste by Environmental Services personnel.

IMPORTANT: The EPA does not allow the accumulation of sharps; including pipettes and pipette tips or other minimum puncture potential sharps, in bags or open containers on a lab bench. All sharps, regardless of their puncture potential, must be accumulated for disposal immediately into an approved sharps container. Although this procedure may, in part, appear overly protective and burdensome it must be followed in order for the university to avoid EPA violations. The EPA has issued notice of violations to the university in the past for failure to follow this requirement.

Please contact EHS at 2215 if you have any questions regarding these procedures.

Chemical Sterilant: A germicide that can destroy all forms of microbial life when adequate exposure conditions are realized. Chemical sterilants are often used as high-level disinfectants when shorter contact times are utilized.

Decontamination: The killing of organisms or removal of contamination after use, with no quantitative implication, generally referring to procedures for making items safe before disposal.

Disinfectant: A germicide that inactivates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms. May not be effective against bacterial spores.

Disinfection: The elimination or destruction of all pathogenic microorganisms. The term has been extensively misused and generally applies to the destruction of any pathogenic vegetative bacteria.

High-level: The elimination or destruction of all microorganisms with the exception of high numbers of bacterial spores.

Intermediate-level: The elimination or destruction of all vegetative bacteria including the *Mycobacteria*, most viruses, and most fungi but does not necessarily kill bacterial spores.

Low-level: The elimination or destruction of pathogenic vegetative bacteria, some viruses, and some fungi but not *Mycobacteria* or bacterial spores.

Germicide: An agent that destroys microorganisms, particularly pathogenic microorganisms.

Sanitization: The process of reducing microbial contamination to an acceptable "safe" level. The process of cleaning objects without necessarily going through sterilization.

Steam Sterilization: Autoclave, the process of sterilization by the use of heated steam under pressure to kill vegetative microorganisms and directly exposed spores. Common temperature and pressure for being effective is 121°C (250°F) at 15 psi (pounds per square inch) over pressure for 15 minutes. Special cases may require a variation of the steam temperature and pressure used.

Sterilization: The complete elimination or destruction of all forms of life by a chemical or physical means. An absolute not a relative term.

Sterilization

Steam Sterilization

The use of steam under pressure is perhaps the most efficient means of sterilization and is widely used in laboratory and medical facilities to sterilize equipment, glassware, and contaminated materials. All pathogenic bacteria, both vegetative and spore forms, are destroyed within twelve minutes of exposure and direct contact to pure steam heat of 121°C (121°F). Most are destroyed within seconds of exposure. Pure steam at a pressure of 15 psi (pounds per square inch), one atmosphere over pressure, corresponds to the temperature of 121°C. Adequate time must be permitted to attain the 121°C for an exposure of at least 12 minutes for all portions of the articles that are being steam autoclaved. Because of the necessity to allow for adequate exposure for all portions of the materials that are being autoclaved it is necessary to increase the minimum exposure time to 15 minutes. The duration of time needed to adequately heat sterilize material will be dependent upon the quantity and type of material being sterilized at one time, the larger the load the longer the time needed to achieve the needed temperatures deep within the load.

The effectiveness of a routine steam sterilizing cycle can be determined by using the appropriate biological indicator (**Appendix C**), ampoules or test strips containing *Bacillus stearothermophilus* spores or a spore enzyme (a-D-glucosidase) based rapid readout test. There are also several chemical indicators that can also provide reliable information. The standard biological indicator that is used in monitoring the effectiveness of steam sterilization are the *Bacillus stearothermophilus* spores because the spores are highly resistant to high temperatures. The use of the spore enzyme test is increasing in popularity because of its ability to provide results within 3 hours of exposure. The use of temperature sensitive autoclave tape can be misleading since the tape is only capable of indicating that a general temperature was reached. It does not indicate how long the material was exposed to the high temperature.

Autoclaved biological indicator samples should be examined for growth following an exposure to an actual autoclave cycle. The presence of growth in a *Bacillus stearothermophilus* sample or the presence of a color or of a fluorescing color change in other indicators after being steam autoclaved indicates that the exposure cycle was not adequate and must be repeated. In addition to the use of a biological indicator for determining the effectiveness of an autoclave cycle it is important that the researcher be aware of any special handling requirements that may be needed to effectively neutralize their cultured microbial agent or contaminated laboratory equipment. The researcher must understand and handle potentially infectious materials accordingly to reduce the potential for exposure.

The types of materials that may be steam sterilized in an autoclave can be varied in form; by shape and size, solid or liquid in composition or a combination of all, and the autoclave must be capable of accommodating for the type of load. The type of load to be autoclaved will determine the type of steam sterilizing cycle to be used; a liquid load requires a slow depressurization to prevent the liquid from boiling over once the autoclave pressure is reduced.

There are a number of different manufactures and different model designs of steam autoclaves. Before using any steam autoclave, the operation instructions for proper use and timing

requirements must be reviewed. Operators of a steam autoclave must remember that a steam autoclave is operated under pressure and at elevated steam temperatures. Failure to review the operational directions can result in improper sterilizing cycle being used, damage to the materials being exposed to the steam heat, damage to the autoclave and potentially serious or fatal injuries of the operator. Personal injuries can result from steam burns and from not allowing the autoclave to depressurize properly. If a steam autoclave is not working properly do not use the unit until it is repaired, contact the responsible person for the unit and inform them of the problem and label the unit "Out of Service".

In accordance with Wright State University policies dealing with the handling of infectious waste materials, infectious waste materials are to be disposed of according to university procedures defined in **Appendix D**, *Infectious Waste Management Guide* of this manual.

Not all materials are capable of being exposed to steam sterilization in an autoclave. For those items that can not be steam sterilized there are other alternatives in the form of gas sterilization or chemical disinfectants that can be used given proper consideration to practicality, the desired level of disinfection and potential hazards associated with handling of the item and the disinfectant.

Gas Sterilization

Ethylene oxide and formaldehyde gases are generally used for gas disinfection as fumigants under controlled conditions. Ethylene oxide and formaldehyde require special handling procedures to minimize potential personal exposure. Both materials are considered to be suspect carcinogens according to OSHA and an occupational carcinogens according to NIOSH.

Ethylene Oxide (CAS #75-21-8)

Ethylene Oxide (ETO) is used primarily as a means of sterilizing materials that are not designed to be exposed to steam sterilization. The use of ethylene oxide on sensitive plastics, medical and biological preparations and other heat sensitive equipment has contributed to revolutionizing developments in the medical field. Early testing found that ethylene oxide was very effective as a killing agent of bacteria, spores, molds and viruses.

Studies that were conducted to identify the method of activation involved in the destruction of exposed microorganisms found that ethylene oxide caused the replacement of a labile hydrogen with an alkyl group on hydroxyl, carboxyl, sulfhydryl, amino and phenolic groups. The alkylation of these compounds in organisms affects cellular function and structure which leads ultimately to inactivation of cellular function and ultimately death.

As effective as ethylene oxide is as a gas sterilizer, it has some major drawbacks that are potentially hazardous that limit its use in a general laboratory environment. Ethylene oxide is a highly flammable and potentially explosive gas. The gas has an explosive concentration range of 3 to 100 percent, and it is listed as a suspect human mutagen and carcinogen. Because of the potential health risks and flammability potentials there are special handling and ventilation requirements that must be used when handling ethylene oxide. Due to the hazards associated with potential exposures OSHA has listed an

exposure limit of 1 ppm for the duration of a work day. Ethylene oxide is a gas at room temperature and is not to be used in the open environment of the laboratory due to its volatility and health affects.

Ethylene oxide sterilizers are specifically designed to either use a mixture of ethylene oxide and carbon dioxide (10:90) or to use 100 percent ethylene oxide. Before an ethylene oxide sterilizer is to be used the unit should be checked for integrity and the operator must be familiar with operational procedures. The exposure time for a sterilization cycle is usually 4 to 6 hours in duration followed by a period of ventilation to allow for thorough dissipation of absorbed gas. The venting of the sterilizer following use is necessary, exposure to the residual material can be damaging to skin and may present a potential fire hazard.

To test for proper operation of an ethylene oxide sterilizer the biological indicator *Bacillus subtilis* var. niger is used. The spores from *B. subtilis* were found to be highly resistant to the effects of exposure to ethylene oxide

If ethylene oxide is being used in the laboratory it is the laboratory supervisor's responsibility to review all relevant safety information in the safe use, handling and disposal of this material and to be certain that others working in the laboratory receive appropriate training and warnings. Contact the Department of Environmental Health and Safety for assistance in assessing the potential for personal exposures and evaluation of laboratory handling procedures.

Formaldehyde (CAS#50-0-0)

Formaldehyde gas is most frequently used in the process of performing space fumigation of a room or of a piece of laboratory equipment that operated with a controlled environment. At the present time the only accepted method available for decontaminating a biological safety cabinet is to use formaldehyde gas. Formaldehyde gas for decontamination of a biological safety cabinet is generated by heating flaked or powdered paraformaldehyde in the presence of an elevated humidity of nearly 65 percent. Paraformaldehyde generates formaldehyde gas when it is depolymerized by heating to 232 to 246°C (450 to 475°F); the depolymerized material reacts with the moisture in the air to form formaldehyde gas. Using a balanced amount of ammonium bicarbonate neutralizes the formaldehyde gas within the biological safety cabinet. Only individuals that have specific training are permitted to decontaminate biological safety cabinets.

In areas where formaldehyde may be used for fumigation it is important to be aware of potential contacts with incompatible materials that could cause the formation of dangerous reaction products. Clear all materials out of an area where formaldehyde may be used to minimize the chance of a possible reaction with incompatible chemicals. Formaldehyde can react violently or explosively when exposed to incompatibles; in the presence of strong oxidizers there is a chance of fire and explosion or when exposed to hydrogen peroxide there is a violent reaction. Most notable however, formaldehyde may combine with hydrochloric acid or hydrogen chloride to form *bis*(chloromethyl) ether (BCME), a carcinogenic compound.

OSHA, NIOSH and IARC recognize formaldehyde as a suspect carcinogen. OSHA has established an exposure limit of 0.75 ppm during a workday. The Department of Environmental Health and Safety can

evaluate work tasks and perform monitoring tests to determine the potential for an occupational exposure.

Chemical Disinfectants

Choosing a Chemical Disinfectant

A variety of concerns must be addressed when choosing a disinfectant for use in a biohazard area. No one disinfectant is universally ideal and the decision as to the optimum disinfectant involves the consideration of factors such as:

Organism susceptibility
Material or surface to be disinfected
Organic load of the material being disinfected
Potential health risks to laboratory personnel
Hazardous properties of the disinfectant (i.e., flammable, corrosive, toxic)
Stability of the disinfectant
pH, temperature and presence of other contaminants in media and water for dilution
Required contact time for effective disinfection
Requirements for disposal of the disinfectant
Cost

Choosing a disinfectant is, therefore, a decision that requires a fairly detailed knowledge of the target organism, a basic knowledge of disinfectants, and careful consideration of the above factors as they apply to the unique potential conditions in which your laboratory will employ the disinfectant. Always consult the product information, the material safety data sheet (MSDS), on a disinfectant before using the material. Appropriate personnel protective equipment is required to be worn when materials are being mixed and used.

For the chosen chemical disinfectant to be affective when used it must be able to make direct contact with the target organism. Environmental factors such as air bubbles, grease, dirt, a dense concentration of microorganisms and the presence of other chemicals (i.e., soaps) can reduce the effectiveness of the disinfectant.

The Halogens

Chlorine

Chlorine is one of the least expensive and most effective disinfectants. The recommended concentration of sodium hypochlorite for "clean surface" disinfection is 200 ppm, representing approximately a 1:250 dilution of household bleach. The CDC recommends a 1:10 dilution of household bleach as the disinfectant of choice for blood spills while many laboratory safety texts recommend the use of undiluted household bleach for biohazard spill containment. These varying recommendations occur primarily because of chlorine's easy inactivation by organic material (serum, blood, proteins, etc.) and the fact that chlorine's disinfectant activity, unlike many of the other disinfectants, increases as the concentration increases.

Of all the disinfectants, chlorine has one of the most extensive ranges of organisms that are susceptibility to destruction under ideal circumstances. All of the vegetative bacteria that have been tested are susceptible to chlorine destruction, including the acid-fast bacteria. Bacterial spores are also susceptible although longer exposure times are generally required. Both enveloped and non-enveloped viruses are susceptible to chlorine inactivation.

One of the main disadvantages of chlorine as a disinfectant is the ease with which it is inactivated by organic material. Materials to be disinfected should be first cleaned to remove the organic material or the concentration of the chlorine must be increased to compensate for the organic material inactivation. Chlorine is also easily inactivated by a variety of metals including copper, zinc, nickel, iron, etc. and the use of chlorine as a disinfectant on these materials requires increased concentrations of chlorine, often resulting in damage to the substrate materials being disinfected.

Chlorine disinfectant solutions are also extremely sensitive to pH and the sensitivity has dramatic implications on the effectiveness of these solutions. Chlorine solutions are most active under slightly acid conditions (pH 6 to pH 7), the activity level decreases rapidly under conditions where the pH goes from a pH 7 to pH 8.5. As the pH of chlorine solutions increases the disinfectant activity levels decreases.

The limited pH range in which chlorine is effective, slightly acid to slightly basic, is also a limiting factor necessitating the use of nonionic detergents or precleaning followed by thorough rinsing.

A number of alternative forms of chlorine exist to use in the form of household bleach (sodium hypochlorite). Chlorine dioxide compounds are high level disinfectants/steriliants that offer somewhat increased activity and resistance to organic inactivation in comparison to household bleach. Chloramine-T and other organic chlorine compounds also offer increased resistance to organic inactivation but at the cost of decreased activity. While these compounds offer specific advantages, household bleach remains one of the best disinfectants available.

Important Information when Considering to Use Hypochlorite Solutions:
Three situations exist where the uses of hypochlorite solutions pose a potential risk to personnel using the compound. First, the addition of acid to hypochlorite solutions will produce a rapid production of toxic chlorine gas. Second, the contact of chlorine solutions with formaldehyde produces the carcinogen bis-chloromethyl ether. Lastly, the heating of chlorine solutions produces the carcinogen trihalomethane. Chlorine solutions, therefore, must never be autoclaved.

Iodine
Iodine-based disinfectants share the same properties as the chlorine-based disinfectants but are somewhat less reactive with substrates and microorganisms. Like chlorine disinfectants, the iodines are effective against vegetative bacteria, acid-fast bacteria, bacterial spores, and both enveloped and non-enveloped viruses although longer contact times are generally required under similar conditions. Most of the iodine-based disinfectants utilized in laboratory and medical situations are combinations of elemental iodine or triiodide with a neutral polymer carrier molecule. These compounds are collectively referred to as iodophors. Iodophors are excellent disinfectants and antiseptics and are extensively used for surgical scrub solutions, hand-washing compounds, and disinfectants for small laboratory objects.

Unlike the elemental chlorine and iodine, however, iodophors are extremely sensitive to concentration and are quite expensive.

Alcohols

Ethyl and Isopropyl Alcohol

Ethanol and Isopropyl alcohol are both excellent disinfectants whose germicidal properties are generally underestimated. Both are rapidly bacteriocidal against vegetative bacterial forms, tuberculocidal, fungicidal, and virucidal. Neither inactivates bacterial spores and isopropyl alcohol fails to inactivate hydrophilic viruses. Both ethanol and isopropyl alcohol should be considered as intermediate-level disinfectants.

One of the most critical factors in the use of alcohols as disinfectants is concentration. The disinfectant properties of both ethanol and isopropyl alcohol rapidly drop at concentrations below fifty percent (50%) and above ninety percent (90%). Peak disinfectant activity occurs at approximately sixty-seven percent (67%) concentration. The recommended concentration for use is sixty - ninety percent (60 - 90%) by volume.

Both ethanol and isopropyl alcohol are volatile and flammable compounds and must only be used with adequate ventilation. Alcohols, in general, are destructive to rubber compounds and to most of the cement and glues used in instruments, especially optics.

Phenolic Compounds

Phenol

Ever since the adoption of carbolic acid by Lister as the first germicide, phenols have been extensively used. Numerous studies, beginning with a study by Kronig and Paul in 1897, have explored the various chemical substitutions and their effect upon germicidal properties. Today, the only phenolic derivatives found in extensive use, as disinfectants are *o*-phenylphenol, *o*-benzyl-*p*-chlorophenol, and *p*-tert-amylphenol. The mode of action of phenolic compounds appears to be a generalized cytoplasmic poisoning at higher concentrations and an inactivation of enzyme systems and cell wall integrity at lower concentrations.

Overall the phenolic derivatives are all characterized by a broad-spectrum of activity against gram-positive and gram-negative bacteria, fungicidal, tuberculocidal, and virucidal activity against lipophilic viruses (enveloped viruses). Phenols have a high tolerance to both organic load and hard water. Their use also results a residual activity on surfaces. Overall, phenolic derivatives are best classified as low- to intermediate- level disinfectants appropriate for general use in noncritical or semicritical areas. They lack sporicidal activity and are ineffective against noneveloped viruses. Phenol should never be used for sterilization purposes.

Phenolic compounds may exhibit dramatic toxic effects. Phenol compounds rapidly penetrate porous compounds and tend to accumulate in the body fat of exposed animals. Reports of phenolic disinfectant induced skin depigmentation, nerve demyelination and skin contact dermatitis that requires personnel using phenolic disinfectant be provided with appropriate protective clothing and equipment.

Two halogenated phenolic derivatives; parachlorometaxylenol (PCMX) and 2,4,4'-trichloro-2-hydroxydiphenol (Triclosan, Irgasan), are commonly used as antibacterial agents in soaps and scrubs as well as preservatives in a number of products. PCMX has become the most widely used antiseptic scrub in surgery and is used as a preservative in products ranging from printing inks to cosmetics to shoe polishes. Triclosan is now commonly used in antibacterial soaps and deodorants as well as being incorporated into plastics as a "permanent" (but questionable) antibacterial.

Chlorhexidine

Discovered during a search for potential anti-malarial drugs, chlorhexidine proved to have a high level of antibacterial activity, low mammalian toxicity, and a strong affinity for binding to skin and mucous membranes, all of which are desirable characteristics for an antiseptic. Chlorhexidine compounds are generally active against gram-positive and gram-negative vegetative bacteria and lipophilic viruses. Many fungi are sensitive to chlorhexidine and acid-fast bacteria are generally inhibited but not killed (bacteriostatic). Bacterial spores are not killed but germination is inhibited while in contact with chlorhexidine.
Chlorhexidine's activity at relatively low concentrations involves a series of related cytologic and physiologic changes culminating in ion leakage from the cytoplasmic membrane and cytoplasmic precipitation. Chlorhexidine's primary advantage over other disinfectants and antiseptic agents involves both its rapid rate of bacteriocidal activity and its strong binding to skin and mucous membranes.

Chlorhexidine is best classified as a low- to intermediate- level disinfectant appropriate for noncritical and semicritical area disinfectant. As an antiseptic, the lack of direct tissue toxicity and the rapidity of action makes chlorhexidine an excellent bacteriocidal skin cleanser and wound cleaning agent.

Quaternary Ammonium Compounds

Quaternary ammonium disinfectants (quats) first appeared in the late 1930's. Since the original introduction, there has been the addition of numerous compounds, blends, different adjunctive agents, etc., making the entire group of quaternary ammonium disinfectants a rather broad group with a variety of activities, advantages, and disadvantages. The major advantages that are common to the group are an inherent surfactant activity, allowing them to also serve as cleansing agents, and a relatively low level of mammalian toxicity. Common disadvantages include a lack of sporicidal activity and a lack of activity against acid-fast bacteria (except for some of the latest generation quats).

The first generation of quaternary ammonium compounds were the standard benzalkonium chloride compounds developed in the 1930's. Substitution of the aromatic ring hydrogen with chlorine, methyl, and ethyl groups resulted in increased activity and the generation of the second generation of quaternary ammonium compounds. The third generation of quaternary ammonium compounds, or the dual quats, were developed in 1955 and represented compounds with superior microbiological activity. Presently, the quaternary ammonium compounds, now polymeric and polysubstituted quaternary ammonium compounds, are in the seventh generation of development. The newest generation of quats possess a wide spectrum of activity with minimal mammalian host damage and are used in pharmaceuticals, ophthalmic solutions, and contact lens solutions, etc.

The antimicrobial activity of quaternary ammonium compounds appears to be by inactivation of critical enzyme systems. Inactivating substances vary dramatically between the generations of quats with the later generations generally much less susceptible to inactivation by extraneous material such as organic load or hard water.

As far as choosing a quaternary ammonium disinfectant, it is critical to read the label directions on the bottle. Organism susceptibilities differ dramatically between different generations of quats and different formulations.

Appendix F
Internet and Available Publications for Biosafety Resources

With access to the world wide web, the internet can be a valuable asset to the researcher or the laboratory associate searching for additional information on a topic of interest or seeking general information. With a few starting points there are a multitude of locations that can be accessed to provide information as well as names of persons to contact. If you have access to the internet here are some locations that can a valuable resource location.

In addition to the resources on the internet there are publications available for review through the university libraries and the Department of Environmental Health and Safety. If there are additional publications that would be of benefit to others and are not listed please forward the information to the Department of Environmental Health and Safety for consideration.

I. Internet Access: (URL's)

A. Academic Institutions:

Duke University, Occupational and Environmental Safety Services (OESO)
www.safety.duke.edu/department/index.html

Florida State University, Environmental Health & Safety
www.fsu.edu/~safety/bio.htm

Michigan Sate University, Office of Radiation, Chemical and Biological Safety (ORCBS)
www.msu.edu

(The) Ohio State University, Office of Environmental Health and Safety, BioSafety Program
www.ehs.ohio-state.edu/biosafety.html

Oklahoma State University, Environmental Health & Safety
www.pp.okstate.edu/ehs

Stanford University Department of Environmental Health and Safety
www.-leland.stanford.edu/dept/EHS/index.html

State University of New York at Buffalo, Occupational and Environmental Services (OES)
http://wings.buffalo.edu/services/fac/oes

University of California San Diego, Environmental Health and Safety
http://www-ehs.ucsd.edu

University of California Santa Barbara Department of Environmental Health and Safety
http://ehs.ucsb.edu/programs/f-programs.html

University of Florida, Environmental Health and Safety, Biological Safety Program
www.ehs.ufl.edu/bio/default.asp

University of Wyoming, Environmental Health and Safety
http://safety.uwyo.edu/ehs

Wayne State University, Office of Environmental Health & Safety
www.science.wayne.edu/~oehs

Yale University, Office of Environmental Health & Safety
www.yale.edu/oehs/ohsp.htm

B. Agencies and Organizations:

Agency for Toxic Substances and Disease Registry (ATSDR)
http://atsdr1.atsdr.cdc.gov.8080/toxfaq.html

American Biological Safety Association (ABSA)
www.absa.org

Biosafety Resources - directs internet access to:
X Risk Group Classification for Infectious Agents (print table or as PDF file)
X MSDS's for Infectious Agents
X Rules, Regulations and Permits (Permits, rDNA, Infectious Agent Rules)
X Requirements for Facilities Transferring or Receiving Selected Agents
X Agencies and Organizations (ABSA, ASM, ATSDR, CDC, FDA, NSF, etc.)
X Biosafety Pages from other Academic Institutions and other organizations
X Laboratory Biosafety Guidelines (*Biosafety in Microbiological and Biomedical Laboratories*, *Use of Biological Safety Cabinets*, *Guidelines for Transport of Infectious Substances and Diagnostic Specimens*, *NIH Guidelines for rDNA*, and *LCDC Canada, Lab Biosafety Guidelines*)
X Online Journals (MMWR, Medscape, Texas A&M Medical Sciences Library)
X Products and Services (available Commercial Training Courses, Certifications and Equipment)
-Guest Book - How to participate in the Biosafety Discussion Listserv (BIOSAFTY)
-What's New
- Information postings from the ABSA (job postings, articles of information, reviews)

Center for Disease Control (CDC)
www.cdc.gov

Extoxnet - Information on Pesticides
http://ace.orst.edu/info/extoxnet

Laboratory Centra for Disease Control - Canada, Office of Biosafety
www.hc-sc.gc.ca/main/lcdc/web/biosafty/index.htm

National Institute of Health (NIH)
www.nih.gov

National Sanitation Foundation - Biological Safety Cabinets
A Listing of Certified Equipment
www.nsf.org/certified/Cabinets.cfm

NSF Field Service Technicians
www.nsf.org/certified/Cabinets.cfm#Accredit

NIOSH (National Institute of Occupational Safety and Health)
www.cdc.gov/niosh

OSHA (Occupational Safety and Health Administration)
www.osha.gov

U.S. Environmental Protection Agency (USEPA)
www.epa.gov

C. Biological Safety Cabinets - Manufacturers with NSF certified cabinets

Baker Co.	www.Bakerco.com/hiband
FormaScientific	www.Forma.com/BCpg1.html
Heraeus*	www.heraeus-instruments.de/home.cfm?=E&uid=55490

*this company is new to the US market, NSF certification is pending

Labconco	www.Labconco.com
NuAire	www.NuAire.com

D. Institutional Biosafety Committees Home Page (Contains site locations for Institutional Biosafety Committees at other institutions)
www.nbiap.vt.edu/ibc-url.html

E. Publications, available on the Internet

Biological Material Safety Data Sheets (MSDS=s) from Health Canada, Health Protection Branch, Laboratory Centre for Disease Control

www.hc-sc.gc.ca/hpb/lcdc/biosafty/msds/index.html

Biosafety in Microbiological and Biomedical Laboratories, 4th (CDC/NIH)

www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Guidelines for Use of Biosafety Cabinets

www.cdc.gov/od/ohs/biosafty/bsc/bsc.htm

Mortality and Morbidity Weekly Report (MMWR) (CDC)

www.cdc.gov/epo/mmwr/mmwr.htm

Office of BiotechnologyActivities (Formerly called: Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

www.nih.gov/od/oba/

F. Search Engines on the Web

Use of search engines such as AOL.COM, HotBot, Lycos, Netscape and Yahoo and by using the key word *biosafety* to obtain hits on locations found within the world wide web. The subsequent search of hits can lead to a variety of sources of information for review.

II. Publications Available for Reference:

Block, Seymore S. (editor), 1991, **Disinfection, Sterilization and Preservation**, 4th ed., Lea & Febiger (Fordham HSL Library) (Call No. QV220 D611 1991)

Fleming, Diane O.; Richardson, John; Tulis, Jerry J.; and Vesley, Donald (editors), 1995, **Laboratory Safety, Principles and Practices**, Second Edition, ASM Press (EHS Office)

Liberman, Daniel and Gordon, Judith G., 1989, **Biohazards Management Handbook**, Marcel Dekkar Inc. (EHS Office)

Journal of the American Biological Safety Association, American Biological Safety Association National Research Council, 1989, **Biosafety In The Laboratory, Prudent Practices for the Handling and Disposal of Infectious Materials**, National Academy (EHS Office)

NSF Listings: Biohazard Cabinetry, Class II Cabinet Certification and Field Certifier Accreditation, July 1995 - current issue

Rayburn, Stephen R., 1990, **The Foundations of Laboratory Safety, A Guide for the Biomedical Laboratory**, Springer-Verlag (EHS Office)

Richmond, Jonathon, editor, 1999, **Anthology of Biosafety, I. Perspectives of Laboratory Design**, American biological Safety Association (EHS Office)

Richmond, Jonathon Y. and McKinney, Robert W., 1999, **Biosafety in Microbiology and Biomedical Laboratories**, 4th edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institute of Health (EHS Office)

Richmond, Jonathon Y. and McKinney, Robert W., 1993, **Biosafety in Microbiology and Biomedical Laboratories**, 3rd edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institute of Health (EHS Office)

Richmond, Jonathon Y., Ph.D. (editor), 1997, **Designing a Modern Microbiological/Biomedical Laboratory, Lab Design Process and Technology**, American Public Health Association (EHS Office)

Ryan, Kenneth J., (editor), 1994, ***Sherris*** **Medical Microbiology, An Introduction to Infectious Diseases**, 3rd edition, Appleton &Lange (EHS Office)

Appendix G
Selection and Proper Use of Biological Safety Cabinets

New Installation Planning:

- Identify the proper type of biological cabinet that is needed for the work that is going to be performed. Consult with the Department of Environmental Health and Safety for assistance in selecting the right cabinet.
- The placement of the biological safety cabinet in the work area is important. Do not place the cabinet near an exit door, in an area of heavy traffic or under air supply ducts for the room. Activities that cause a disruption of the general airflow will affect the ability of the cabinet to perform properly.

Operational Limitations of Biological Safety Cabinets:

- Biological safety cabinets are not to be used as storage locations of equipment.
- A Type II Class A biological safety cabinet is not to be used with volatile chemicals. The Class A cabinet vents directly back into the room.
- For proper performance and to provide the protection that the cabinet is designed to provide the cabinet must be maintained and serviced as necessary. Failure to maintain the cabinet can result is situations where either, or both, product or personal protection is compromised.

General Procedures:

- Do not use the top of a cabinet for storage. The exhaust filter can be easily damaged by materials falling onto the exhaust filter.
- Do not use the cabinet for long term storage of materials. Keep the inner work surface clear of materials between procedures.
- The biological cabinet is to be field certified at least on an annual basis. The cabinet will meet the NSF Standard 49 (Class II Biohazard Cabinetry) or the manufacturer's specifications for proper operation.
- Identify the chemicals that are going to be used and obtain a Material Safety Data Sheet (MSDS) for those items. Review the information contained in the MSDS with personnel before the material is put into use. The MSDS must be readily available for review.
- Identify any special precautions associated with biological agents that are going to be manipulated. Have written procedures for special handling and cautions.
- Identify and establish written procedures for the use of the biological safety cabinet and for spill containment.
- Maintain a use log of organisms or agents used in the cabinet. The log will also contain a record of all maintenance performed. The log will be a permanent record for the cabinet.

Preparation for Use:

- If a UV light is present in the cabinet, the UV light is to be turned OFF whenever people are going be working in or in the area of the cabinet.
- Check the drain valve to make sure that it is closed. To be closed the valve handle should be parallel to the bottom of the cabinet (at a right angle to the valve).
- If the cabinet is OFF, turn ON the blower to initiate the airflow within the cabinet.
- If the front sash is movable, set the sash at the proper operational height for the cabinet.
- The work surface should be disinfected with an appropriate disinfectant that will kill the microorganisms that are likely to be present.
- Allow the cabinet to operate for 5 minutes to establish air flow patterns.
- Check the cabinet for proper operation; ie., check the magnehelic gauge for reference.

Note: *If the cabinet is NOT operating properly, as designed, DO NOT use the cabinet until the problem has been corrected. Failure to have an operational problem corrected can result in the exposure of the operator to the agents that are being manipulated in the cabinet with the potential of serious consequences.*

- Place only needed equipment inside the cabinet, do not block or obstruct the front or rear ventilation grilles of the cabinet.
- Establish work zones within the work surface area to minimize cross contamination, clean materials should be upstream of contaminated areas.
- Do not place upright pipette collectors in the cabinet use a flat collection tray with a cover to contain used pipettes.
- A biohazardous material collection bag should be placed inside the cabinet to contain biohazardous waste material. Passing generated biohazardous waste material outside of the cabinet to a collection bag may cause the operator to be exposed to aerosolized infectious materials.

Working in the Cabinet:

- Wash hands before starting to work in the cabinet.
- The use of PPE (personal protective equipment) is required even while working in a biological safety cabinet. ANSI approved safety glasses or goggles are to be worn, a lab coat with appropriate gloves. Wearing of shorts and sandals are discouraged while working.
- No eating, drinking or chewing of gum while working in a biological safety cabinet.
- Only one person is to work in a cabinet at a time.
- Do not use volatile organic compounds in a Class II Type A biological safety cabinet.
- Adjust seating height so that the operator's arms do not rest on the front edge or block the front grille.
- Do not use open flames in a biological safety cabinet. If a flame source is needed use a touch-o-matic type flame source.

- Movements in and out of the cabinet while working should be straight in and out and should be kept to a minimum, avoid sweeping movements that will cause a disruption of the airflow profile within the cabinet.
- Use the biohazardous material collection bag inside of the cabinet to contain biohazardous waste and to reduce to passing of the operator's arms through the clean zone of operation.

Post Procedure Clean Up:

- Potentially contaminated equipment within the cabinet must be surface disinfected before being removed from the cabinet.
- Use an appropriate disinfectant on the work surface once cleared of equipment.
- After all potentially contaminated equipment has been disinfected, remove gloves and wash hands.
- If the cabinet blower is going to be turned OFF, allow the cabinet to run for several minutes to perform a final purge of the cabinet.
- If a UV light is activated, pull the front sash down to close off the front opening of the cabinet. DO NOT activate a UV light if the sash is fixed in place and there are going to be people working in the vicinity of the cabinet.
- Do not store materials or supplies inside of a biological safety cabinet.