WAYNE STATE UNIVERSITY

Biosafety Manual

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Wayne State University
Biosafety Manual

Introduction

The goal of this manual is to provide policies and procedures that when implemented, will reduce risks to the Wayne State University community from biological agents, including pathogenic organisms and derived toxins. These policies and procedures reflect current biosafety regulatory expectations, as well as currently accepted biosafety practices, and are designed to safeguard personnel, facilities, and the environment without inhibiting research activities. Principal Investigators and laboratory workers are expected to be familiar with the requirements of this Manual, and to implement these requirements in their laboratory operations.

The hazards present in any particular laboratory are rarely limited to biological agents; chemical hazards are almost always of concern, and radiological hazards are also often present. Consequently, biosafety should not be approached separately from other laboratory hazards, but be viewed as just one component of a total laboratory safety program. Guidance on chemical and radiological hazards can be found in the WSU Chemical Hygiene Plan and WSU Radiation Safety Manual, respectively. In an effort to facilitate distribution and future revision, this Manual is provided in electronic format (OEHS will provide hard copies only upon request). Laboratories should print a hardcopy directly from the OEHS web site (http://www.oehs.wayne.edu).

Success of the WSU Biosafety Program requires a team effort involving the Institutional Biosafety Committee, academic departments, **Principal Investigators, laboratory workers, and the Office of Environmental Health and Safety. Principal Investigators are responsible for the health and safety of personnel who work under their supervision and occupy their laboratory space.** Consequently, they are in a unique position to positively influence the implementation of the safe work practices contained in this manual. University administration, the Institutional Biosafety Committee, and the Office of Environmental Health and Safety, endorse this manual and encourage active participation in maintaining high standards of biosafety at WSU.

Chapter 1

Purpose, Scope, and Responsibilities

Purpose

The purpose of the Wayne State University's Biosafety Manual is to minimize risks to personnel, facilities, and the environment while handling biological agents during teaching, research, and clinical activities at WSU. The work practices, procedures and policies specified in this manual are based on current accepted biosafety practices. Implementation of these measures will reduce the likelihood that an incident involving a biological agent will occur, and will fulfill regulatory biosafety expectations. Laboratory microbiological work usually involves exposure not only to biological hazards, but to chemical and radiological hazards as well. Consequently, this manual should be used in conjunction with the WSU Chemical Hygiene Plan and the WSU Radiation Safety Manual, as appropriate.

Scope

This manual applies to all WSU activities involving biological agents. All WSU faculty, staff, students, visitors, and employees of industry partners when working on WSU sponsored projects or at WSU facilities, are included in the scope of this manual.

Biological agents include all infectious organisms (bacteria, chlamydia, fungi, parasites, prions, rickettsias, viruses, etc.) that can cause disease in humans, or significant environmental or agricultural impact, and toxins derived from such organisms. Additionally, recombinant DNA; human or non-human primate tissues, fluids, cells or cell culture; transgenic plants or animals; and work with animals known to be reservoirs of zoonotic diseases are wholly or partly covered by the procedures and policies in this manual.

Responsibilities

The responsibility for biosafety at WSU is a team effort requiring the direct involvement of the WSU Institutional Biosafety Committee, the WSU Biosafety Officer and Office of Environmental Health and Safety (OEHS), Principal Investigators (PIs), and laboratory workers.

WSU Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) develops policies and provides leadership with the goal of reducing risks to the WSU community due to biological agents. The IBC is composed of at least five members that collectively represent experience and expertise in a wide range of biosafety areas applicable to WSU activities. At least two members of the IBC must be from outside the WSU community (not directly affiliated with WSU). Non-committee faculty or staff with special expertise will be asked to advise the IBC as appropriate. A current list of IBC Members is available here:
www.oehs.wayne.edu/biosafety/committee.php

Responsibilities of the IBC include:

1. Developing biosafety policies applicable to WSU activities, including work practices, biohazardous waste, and medical surveillance of personnel.
2. Reviewing and approving new research proposals in accordance with CDC/NIH guidelines.
3. Setting required containment levels for research projects. Generally, the biosafety levels (BSLs) established by the CDC and NIH will be used as the level of containment; however, the IBC can increase or decrease the level of containment according to the specific circumstances of the project.
4. Developing design specifications and criteria for containment facilities.
5. Investigating significant violations of WSU biosafety procedures or policies, and significant accidents or illnesses involving Biological Agents. If appropriate, the IBC will recommend disciplinary action to the proper WSU officials.

Biosafety Officer

The WSU Biosafety Officer (BSO) is responsible for providing guidance on safe handling of biological agents and overall management of the Biosafety program. The BSO is a member of the IBC. Specific responsibilities of the BSO include:

1. Providing technical advice to the IBC and PIs on biosafety protocols.
2. Developing emergency response plans for accidental spills and personnel contamination, and investigating incidents involving biological agents.
3. Making periodic inspections of laboratories to assess biosafety issues.
4. Keeping the IBC informed of pertinent biosafety issues and program status.
5. Providing general biosafety training for WSU personnel on a regular basis.

Principal Investigators

Principal Investigators (PIs) are responsible for the health and safety of all personnel in their laboratory. Specific responsibilities of the PI include:

1. Ensuring that specific laboratory hazards are effectively communicated to laboratory staff, and controls are in place to minimize risks associated with these hazards.
    a. Developing laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory. Templates for SOPs are available here.
    b. Ensuring that engineering controls are available, are in good working order, and are used appropriately to minimize exposure to biohazardous agents.
    c. Ensuring that appropriate personal protective equipment is available and used by laboratory personnel.
2. Ensuring that all laboratory personnel receive general Biosafety training conducted by OEHS, as well as specific training on the hazards, procedures, and practices relevant to the laboratory they are working in. All training must be documented and records maintained.
3. Notifying the IBC and obtaining prior IBC approval for work involving biohazardous material as specified in this manual (see Chapter 2).
4. Ensuring that laboratory workers are provided immunizations and medical surveillance prior to exposure to biohazardous agents as appropriate (based on current recommendations of the Centers for Disease Control and Prevention, and IBC recommendations).
5. Notifying the BSO of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment.
6. Ensuring that biological agents are disposed of as outlined in this manual.
7. Ensuring that biohazardous materials to be transported are packaged and shipped in accordance with regulations.
8. Ensuring that an accurate inventory of biological agents is maintained.
9. Ensuring that periodic assessments of the laboratory are conducted to self-identify health and safety weaknesses, and that identified weaknesses are remedied in a timely manner.

Laboratory Workers

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. An incident caused by one laboratory worker can have a widespread effect on others. Specific responsibilities include:

1. Following procedures and practices established by the University and the laboratory.
2. Using accepted good laboratory practices to minimize exposures to biological agents, and to avoid other incidents (such as fire, explosion, etc.).
3. Attend biosafety and other laboratory safety training as required.
4. Report unsafe laboratory conditions to the PI, OEHS, or other responsible party.
5. Utilize control measures such as biological safety cabinets and personal protective equipment to prevent exposure to biological agents, and contamination of personnel and facilities.

Chapter 2

Approval of Research Projects

Projects Requiring Approval

All projects involving biological agents must be reviewed and approved by the Institutional Biosafety Committee (IBC) prior to commencement of the work. Additionally, work involving recombinant DNA; human or non-human primate tissues, fluids, cells or cell culture; and transgenic plants also require prior approval from the IBC. Principal Investigators must submit the appropriate Biological Agent User Application Form (BAUA) to the IBC in order to initiate the approval process.

Biological Agents

All work involving biological agents must be reviewed by the WSU Institutional Biosafety Committee (IBC) for adherence to NIH/CDC biosafety guidance published in the latest edition of Biosafety in Microbiological and Biomedical Laboratories as well as WSU policies and current biosafety practice.

Biosafety Level 1 (BSL1) and Animal Biosafety Level 1 (ABSL1)
Organisms in this category are not known to cause disease in healthy human adults. IBC approval is not required for use of these organisms.

Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2)
All work involving biological agents classified as BSL2 must be reviewed by the IBC. Containment levels, facility requirements, and work practices will generally follow NIH/CDC guidance; however, the IBC can raise or lower these requirements as appropriate.

Principal Investigators are required to submit a BAUA to the IBC in order to initiate the approval process.

Biosafety Levels 3 and 4 and Animal Biosafety Levels 3 and 4
Projects involving BSL 3 or 4 organisms are prohibited at WSU.

Recombinant DNA

As a condition of funding from the National Institutes of Health (NIH), all research at WSU involving recombinant DNA must be conducted in accordance with the most current version of Guidelines for Research Involving Recombinant DNA Molecules available at: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html. PIs are required to make an initial determination of the required biological and

physical containment required. The approval level required for the proposed research is dependent on the NIH category to which the work corresponds. Prior approval by the IBC is required for all proposed experiments involving recombinant DNA, including those exempt from NIH Guidelines. Principal Investigators must submit a BAUA to the IBC in order to initiate the approval process. The following paragraphs summarize experiments covered by the NIH Guidelines; refer directly to these guidelines for a more detailed description of experiments and specific requirements.

Experiments requiring IBC approval, RAC review, and NIH approval (NIH section III-A)

Experiments involving the deliberate transfer of a drug resistance trait to microorganisms that do not acquire the trait naturally, where such acquisition could compromise the use of the drug to control disease in humans, veterinary medicine, or agriculture are included in this category. These experiments are considered “Major Action” and require review by the Recombinant DNA Advisory Committee (RAC) at NIH, and specific approval by NIH, prior to initiation. Additional information on the Office of Biotechnology Activities (OBA) and the RAC can be obtained at: http://oba.od.nih.gov.Approval by the IBC is required prior to initiation of the experiments.

Experiments requiring IBC and NIH approval (NIH section III-B)
Experiments in this category include the cloning of genes encoding toxic molecules with an LD50 for vertebrates less than or equal to 100 ng/kg. This includes microbial toxins such as botulinum toxins, tetanus toxins, and diphtheria toxin. These experiments cannot be initiated without submission of relevant information on the proposed experiment to the OBA. IBC approval is required prior to initiation of the experiments.

Experiments requiring IBC and RAC approval, with NIH registration (NIH section III-C)

These experiments involve the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into humans (human gene transfer). Prior to initiation of laboratory work, these experiments must be approved by the IBC, the RAC, and be registered with the OBA.

Experiments requiring IBC approval (NIH section III-D)
This category includes whole animal or plant experiments, as well as experiments involving DNA from Risk Group 2, 3, or 4 agents. These experiments must be approved by the IBC prior to initiation.

Experiments using Risk Group 1 agents (NIH section III-E)
Experiments in this category are low risk and can be conducted using BSL1 containment. Examples include experiments in which all components are derived

from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes. IBC approval is required prior to initiation of the experiments.

NIH exempt experiments (NIH section III-F)
The following recombinant DNA (rDNA) experiments are included in the NIH Guidelines as “exempt experiments”:

Section III-F-1. Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C it is not exempt under this Section.

Section III-F-2. Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.
If your work is not in one of the above categories, please select the appropriate category from those listed below.

Section III-F-3. Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

Section III-F-4. Those that consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means.

Section III-F-5. Those that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III-F-6. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), Major Actions). See Appendices A-I through A-VI, Exemptions under Section III-F-6--Sublists of Natural Exchangers, for a list of natural exchangers that are exempt from the NIH Guidelines.

Section III-F-7. Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

Section III-F-8. Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), Major Actions), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, Exemptions under Section III-F-8 for other classes of experiments which are exempt from the NIH Guidelines.

Amendments and Terminations
PIs wanting to revise a current research project are required to submit a revised BAUA to the WSU Biosafety Officer. Changes involving modification of biological agents, significant procedural changes, or modifications that increase the risk of the project must be approved by the IBC prior to implementing the change. The PI is required to notify the WSU Biosafety Officer (in writing) when a project is completed or is no longer active.

Annual Updates

PIs are required to verify and update approved projects on an annual basis. The purpose of the annual update is to allow the PI to verify continuance of the project, discontinue the project, or amend the BAUA. Significant modifications require IBC approval.

Chapter 3

Biosafety Regulations and Guidelines

There are several local, state, and federal agencies that either regulate or provide guidelines covering the use of biological agents. A summary of these regulations and guidelines are provided below. Copies of these documents can be obtained from OEHS.

1. Centers for Disease Controls and Prevention (CDC) and the National Institutes of Health (NIH): Biosafety in Microbiological and Biomedical Laboratories (BMBL). This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety and is the basis for this manual.

2. National Institutes of Health (NIH): Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). This document provides guidelines for constructing and handling recombinant DNA molecules (rDNA), and organisms containing rDNA. Although these guidelines are not subject to regulatory enforcement, institutions that receive any NIH funding for rDNA research are required to comply with these guidelines as a condition of funding. This document requires the establishment of an Institutional Biosafety Committee with the authority to approve proposed rDNA research using the NIH Guidelines as a minimum standard.

3. Occupational Safety and Health Administration (OSHA): Bloodborne Pathogens. This regulation covers occupational exposure to human blood and other potentially infectious material. OSHA specifies a combination of engineering controls, work practices, and training to reduce the risk of infection. Personnel potentially exposed to human blood and other potentially infectious material must be offered immunization against Hepatitis B and receive annual training. Personnel who work with HIV or Hepatitis B in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens.

4. Centers for Disease Control and Prevention (CDC): Additional Requirements for Facilities Transferring or Receiving Select Agents. This regulation requires that institutions that transfer or receive certain biological agents and toxins be registered and approved by the CDC. WSU laboratories that transfer or receive these agents must be included on the larger WSU registration. Laboratories must comply with the BMBL and the OSHA Laboratory Standard and the WSU Chemical Hygiene Plan). Each transfer of a Select Agent must be accompanied by a specific CDC form (EA-101) that requires OEHS signature (as Responsible Facility Official) and serves to document the chain of custody. Accurate inventory records of Select Agents, including transfers, must be maintained. Additional information is also available in Chapter 12 of this document.

Chapter 4

Biosafety Principles

Containment

Laboratory biosafety practices are based on the principle of containment of biological agents to prevent exposure to laboratory workers and the outside environment. Primary containment protects the laboratory workers and the immediate laboratory environment from exposure to biological agents. Primary containment is achieved through good microbiological technique and the use of safety equipment and personal protective equipment. Secondary containment protects the environment outside the laboratory, and is provided by facility design and operational procedures.

Laboratory Practice and Technique

The use of good microbiological technique is the most important element of containment. Personnel working with biological agents must be aware of hazards, and must be trained to safely handle and dispose of these materials. Although we are all responsible for our own safety, the Principal Investigator has ultimate responsibility for ensuring that persons working in their laboratory are adequately trained.

This Biosafety Manual has been developed to provide general policies and procedures when working with biological agents at WSU. Each individual laboratory must supplement this manual with laboratory specific policies, procedures and training that will minimize the specific risks present in the laboratory.

Safety Equipment

Safety equipment includes biological safety equipment, safety centrifuge cups, and other engineered controls designed to minimize exposure to biological agents. Biological safety cabinets (BSCs) are the most important safety equipment for protection of personnel and the laboratory environment, and most BSCs also provide product protection. Safety equipment is most effective at minimizing exposure when workers are trained on the proper use of such equipment, and the equipment is regularly inspected and maintained.

Personal Protective Equipment

Personal protective equipment includes safety eyewear, lab coats, and gloves, and is used to supplement the containment provided by laboratory practices and safety equipment. Personal protective equipment is considered the least desirable containment method since its failure results in direct exposure of personnel to the biological agent.

Facility Design

Facility design features include physical separation of laboratories from public access, specially designed ventilation systems (to prevent airborne biological agents from migrating outside the laboratory), and autoclaves. These design

features protect personnel working outside the immediate laboratory, as well the outside environment

Biosafety Levels

The CDC/NIH has developed four biosafety levels that describe laboratory practices and techniques, safety equipment, and facility design features recommended for work with specific infectious organisms. Descriptions of the biosafety levels, as well as assigned biosafety levels for specific organisms, are contained in the CDC/NIH document, Biosafety in Microbiological and Biomedical Laboratories (BMBL). The recommended biosafety level for an organism represents conditions under which the agent can normally be handled safely; however, specific circumstances may dictate that the recommended conditions be raised or lowered. The four biosafety levels are summarized below:

Biosafety Level	Agents	Practices	Safety Equip	Facilities
1	Not known to cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top, sink required
2	Associated with human disease, hazard: auto-inoculation, ingestion, mucous membrane exposure	BSL-1 practice plus: ▪ Limited access ▪ Biohazard warning signs ▪ Sharps precautions ▪ Biosafety manual	*Primary barriers:* Class I or II BSCs or other containment used for manipulations of agents that cause splashes or aerosols of infectious materials; PPE: lab coats; gloves; eye/face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: ▪ Controlled access ▪ Decontamination of all waste ▪ Decontamination of lab clothing before laundering ▪ Baseline serum	*Primary barriers:* Class I or II BSCs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: ▪ Physical separation from access corridors Self-closing, double door access ▪ Exhausted air not recirculated ▪ Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agent with unknown risk of transmission	BSL-3 practices plus: ▪ Clothing change before entering ▪ Shower on exit ▪ All material decontaminated on exit from facility	*Primary barriers*: All procedures conducted in Class III BSCs or Class I or Class II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: ▪ Separate building or isolated zone ▪ Dedicated supply/exhaust vacuum, and decon systems ▪ Other requirements outlined in BMBL

Source: http://www.cdc.gov/biosafety/publications/bmbl5/

for a more complete description of the four biosafety levels, as well as recommended biosafety levels for specific organisms.
In addition to the four biosafety levels described above, there are also four biosafety levels for work with infectious agents in vertebrate animals. For a complete description of the animal biosafety levels, consult the BMBL.

Routes of Transmission

Skin and Mucous Membrane Contact
Low energy procedures such as decanting of liquids, pipetting, removal of screw caps, vortex mixing, streaking agar plates, inoculation of animals, can result in the generation of infectious droplets, as well as result in direct contact with infectious material. Includes eye contact as a route of exposure.

Ingestion
Mouth pipetting presents the highest risk for ingestion of infectious material. Splashing of material into the mouth, and indirect oral exposure through touching the mouth with contaminated hands, and eating and drinking in the lab can also result in ingestion of infectious material.

Percutaneous Inoculation
Use of syringes and needles are considered the greatest risk of exposure through inoculation. Inoculation can also occur as a result of cuts and scratches from contaminated items, and animal bites.

Inhalation
Many procedures have the potential for generation of respirable aerosols, including: sonication, centrifugation, “blowing out” of pipettes, heating inoculating loops, and changing litter in animal cages.

Chapter 5

Laboratory Biosafety Practices

Basic Laboratory Practices

The following prudent biosafety practices are recommended by the National Academy of Sciences. Although these practices may be considered "common sense" and overly simplistic by experienced researchers, strict adherence to these basic principles will greatly reduce the risk of laboratory acquired infection.

Biosafety Practice	Routes of Exposure Blocked
1. Do not mouth pipette	Inhalation, ingestion, skin and mucous membrane contact
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols	Inhalation, skin and mucous membrane contact
3. Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives; dispose of sharps in leak- and puncture-proof containers	Percutaneous, inhalation
4. Use lab coats, gloves, safety eye wear, and other personal protective equipment	Skin and mucous membrane contact
5. Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents	Skin and mucous membrane contact
6. Decontaminate work surfaces before and after use, and immediately after spills	Skin and mucous membrane contact Ingestion,
7. Do not eat, drink, store foods, or smoke in the Laboratory.	Skin and mucous membrane contact

Biological Hazard Information

Laboratory workers must be knowledgeable of the hazards associated with the biological agents present in the laboratory, and have hazard information available to them. The following are sources of hazard information for biological agents.

Microbial Agents

1. Biosafety in Microbiological and Biomedical Laboratories (BMBL): available from the CDC and at http://www.cdc.gov/biosafety/publications/bmbl5/

2. The Canadian Laboratory Centre for Disease Control (LCDC): The LCDC maintains Material Safety Data Sheets for microbial agents on its web site at http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php.

Toxins

Isolated biological toxins are chemical hazards, although many such toxins produce adverse effects at doses significantly below that of "traditional" laboratory chemicals. Laboratory use of isolated toxins falls under the WSU Chemical Hygiene Plan, and Safety Data Sheets (SDSs) must be available in the lab.

1. SDSs for the specific toxin should be received from the vendor upon receipt of the toxin. SDSs may also available through the OEHS web site http://www.oehs.wayne.edu.

2. Toxicology textbooks such as Casarett's and Doull's Toxicology are also good sources of hazard information for toxins.

Written Standard Operating Procedures

This manual, in combination with the referenced CDC/NIH publications Biosafety in Microbiological and Biomedical Laboratories and Guidelines for Research Involving Recombinant DNA Molecules, provides general standard operating procedures (SOPs) for working with biological agents. However, since these SOPs cover relatively general topics, individual laboratories are required to develop laboratory specific SOPs that cover the biosafety concerns and laboratory procedures for that particular laboratory. For example, laboratory specific SOPs should address safe manipulation of specific organisms, specific exposure control methods, and specific decontamination and waste handling requirements. There is no standard format required for SOPs and laboratories are encouraged to use any format that effectively conveys the biosafety information (including use of pictures and illustrations). The laboratory specific SOPs do not need to duplicate the more general SOPs contained in this manual or the CDC/NIH documents, but should supplement these other documents.

Prevention of Aerosols and Droplets

Handling of liquids or dry powders is likely to generate aerosols or droplets. Procedures such as centrifuging, mixing, and pipetting that involve high energy tend to produce respirable aerosols that stay airborne for extended periods and are small enough to be inhaled. Low energy procedures including opening containers and streaking plates produce droplets that settle quickly on surfaces, skin, and mucous membranes.

Biological Safety Cabinets

Procedures involving infectious material should be performed inside a biological safety cabinet (BSC) whenever possible. A properly operating, properly used BSC (see Chapter 8) will contain any aerosols and droplets generated during handling of infectious agents.

Pipetting

Do not mouth pipette! Always use a mechanical pipetting device. Pipettes should be drained gently with the tip against the inner wall of the receiving vessel and liquid should not be forcibly expelled from the pipette.

Blending

Use a safety blender that has leak proof bearings and a tight fitting lid with a sealable gasket.

Centrifugation

The potential for contamination and infection is high if liquid and aerosol is released during centrifugation. Sealed centrifuge buckets, or safety cups should be used to prevent release of liquid and aerosol. If sealed buckets or safety cups are not obtainable, it is recommended that the centrifuge chamber be evacuated before the centrifuge is opened. Some centrifuges have an available access port that will allow evacuation of the chamber using a vacuum pump (use an in-line disinfectant trap and/or HEPA filter to protect the pump from contamination) and tubing attached to a port. Ultracentrifuges operate under vacuum and should contain an in-line HEPA filter between the chamber and the vacuum pump.

Inoculating Loops

Flaming inoculating loops can result in spatter and release of aerosols and droplets. Use of an electric micro-incinerator will effectively control spatter resulting from sterilization of inoculating loops.

Use of Absorbent Materials

Work surfaces should be covered with absorbent paper or “diaper” sheets to collect splashes and drips, and minimize the spread of contamination. The absorbent paper should be changed at the end of the laboratory procedure as part of the final cleanup, or at least daily during use.

Personal Protective Equipment

Although not a substitute for use of BSCs and good laboratory practices, personal protective equipment (PPE) is considered a primary barrier to infectious agents and proper use will reduce the likelihood of infection. PPE is the least desirable exposure control method since its failure results in direct exposure to the agent. PPE is most effective when used to supplement primary control Methods, such as; biological safety cabinets, safety centrifuge cups, and other containment devices.

Laboratory Coats

Laboratory coats protect street clothes against chemical and biological spills, and provide additional body protection. Laboratory coats made of 100% cotton are flame resistant and nonreactive to many chemicals. Generally, a 100% cotton lab coat is recommended over polyester-cotton blends. The wearing of lab coats is considered to be standard microbiological practice for BSL 1 and 2 laboratories. For BSL 3 laboratories, CDC/NIH guidelines recommend solid-front or wrap-around gowns or suits, rather than front-buttoning lab coats. It is good laboratory practice to remove lab coats or gowns before leaving the laboratory to minimize the spread of contamination outside the laboratory. Lab coats should be left in the laboratory and must not be taken home for washing.

Gloves

Gloves are available that provide protection against a variety of hazards, including infectious agents, chemicals, and radioactive material. Unfortunately, there is no single glove type that provides adequate protection for all hazards (or even all chemicals).

Standard latex examination type gloves provide protection against microbiological hazards, including human blood and body fluids. Latex gloves do not generally provide adequate protection against liquid chemicals; additionally, many people develop latex allergies as a result of wearing latex gloves. Thin nitrile gloves are an alternative to latex examination gloves that provide similar dexterity and increased chemical resistance. Nitrile gloves still provide protection against microbiological hazards, but without the latex allergy hazard. Although thin nitrile gloves generally provide better chemical protection than latex gloves, they are not considered to be chemical resistant gloves. For instances where chemical contact is likely or cannot be tolerated due to high toxicity, consult the

WSU Chemical Hygiene Plan or contact OEHS (57)7-1200 for recommendations concerning chemical resistant gloves.

Glove selection for protection against radioactive materials should be based on the resistance of the glove to the liquid solvent that the radioactive material is contained in. Essentially any rubber-type glove material will provide protection against dry chemicals or radioactive material.

Contamination control requires that gloves be removed prior to exiting a BSC or touching non-contaminated laboratory areas and equipment (such as clean areas, phones, computers, door knobs, etc.). Always check gloves for pinholes prior to use and wash hands after removing gloves.

Eye and Face Protection

Safety glasses, goggles, and face shields provide protection against chemical reagents and disinfectants. Additionally, they also prevent infection that can result from the splashing of pathogenic organisms in the eye. Normal prescription eyeglasses are not safety glasses and do not provide adequate eye protection for laboratory operations. Further guidance on the use of protective eye and face wear for chemical hazards can be found in the WSU Chemical Hygiene Plan. Microbial infection can occur as a result of splashes to the eye. Goggles with indirect venting provide a good barrier against such splashes. A face shield can be worn in addition to goggles (face shields do not provide adequate eye protection by themselves) to provide protection against splashes to the face and mouth.

Respiratory Protection

Certain laboratory and clinical situations require respiratory protection to prevent inhalation of infectious agents. Regulations, as well as good safety practice, require that personnel be medically evaluated, specifically trained, and fit tested prior to wearing respiratory protective equipment. Contact OEHS (57)7-1200 if respiratory protective equipment is required or if there are questions about the respiratory protection program.

Storage and Labeling of Biological Agents

Biological agents must be stored using double containment. Both the primary and secondary containers must be durable and leak proof so as to prevent accidental exposure. Primary containers must be clearly labeled as to the identity of the agent and should include the universal biohazard symbol (see below) as physical space on the container permits. At a minimum, secondary (or outside) containers must include the universal biohazard symbol (identity of contents is also desirable). Freezers, refrigerators, and other storage areas must also be labeled with the biohazard symbol; exceptions to this policy will be considered on an

individual basis by the IBC. Waste, and contaminated equipment or other objects to be decontaminated must also be labeled with the Biohazard Symbol.

Universal Biohazard Symbol: The OSHA Bloodborne Pathogen Standard specifically requires that containers of human blood or other potentially infectious material (OPIM), contaminated waste, and refrigerators, freezers, and other storage containers used to store or transport blood or OPIM, be labeled with the universal biohazard symbol (fluorescent orange or orange-red).

Biohazard Labels and Signs: Signs must be posted at or on the access doors indicating that biological agents are used within the room. The sign must include the universal biohazard symbol, the name of the agent(s) present, any specific entry requirements (such as personal protective equipment or immunization), and the name and telephone number of the PI and/or other responsible person(s). The following areas require posting:

1. Entrances to laboratories and animal rooms that use agents classified as

2. Cages or animal rooms used for housing animals infected with BSL2 or BSL3 agents.

Chapter 6

Laboratory Training

Training is required for all laboratory workers (faculty, staff, students, and visiting scientists) at WSU. The exact training required for a particular person will depend on the hazards to which he or she is exposed. OEHS conducts laboratory safety training in a modular format that includes the following topics: chemical hygiene, hazardous waste, chemical spill response, bloodborne pathogen awareness, and biosafety. Since virtually every laboratory worker utilizes chemicals, chemical hygiene, hazardous waste, and chemical spill response are required for all laboratory workers. The awareness level bloodborne pathogen training discusses general topics and exposure prevention and is recommended for all laboratory workers. It is the responsibility of the Principal investigator (PI) or Laboratory Supervisor to ensure that all personnel receive training that is appropriate for their job duties and exposure potential.

General Biosafety Training

OEHS offers a general Biosafety Training class that is specific to each laboratory, regulatory requirements, the WSU Biosafety Program, general biosafety work procedures, and biohazardous waste disposal. WSU laboratory workers who work with biological agents are expected to attend this course; however, individual departments can conduct equivalent training on their own if they desire (all training must be documented). Workers must receive training prior to beginning laboratory work with biological agents. Workers are only required to attend this general biosafety training once, refresher training is not required. OEHS generally offers this training at the beginning of semesters but special classes can be scheduled for groups.

Bloodborne Pathogen Training

OEHS also offers bloodborne pathogen training for people who have potential occupational exposure to human blood, or other body fluids. It is the position of the Centers for Disease Control and Prevention (CDC) and OSHA that all cell lines of human origin be considered potentially infected with bloodborne pathogens, and that these materials be handled using BSL2 containment and procedures. Consequently, all people who work with human cell lines are considered to have potential exposure to bloodborne pathogens, and they are required to be in the Bloodborne Pathogen Program and attend bloodborne pathogen training. Personnel in the Bloodborne Pathogen Program are required to receive refresher training on an annual basis.

HIV/HBV Laboratory Training

Personnel who work in research laboratories that culture, produce, or otherwise perform microbiological manipulation of human immunodeficiency virus (HIV) or

hepatitis B virus (HBV) must receive additional training beyond the standard bloodborne pathogen training. Prior to working with HIV or HBV, laboratory workers must demonstrate proficiency in standard microbiological techniques, and in the practices and techniques specific to the laboratory. Additionally, workers must have prior experience in handling human pathogens before working with HIV or HBV. Personnel who do not have experience with human pathogens must be trained in the laboratory before working with HIV or HBV. Initial training must not include the use of infectious agents, rather training and work activities should be progressive as proper techniques are demonstrated. Workers are permitted to handle infectious agents only after demonstrating proficiency to the satisfaction of the Laboratory Supervisor. Although this specialized laboratory-specific training is the responsibility of the Laboratory Supervisor, the training should be coordinated with the Biosafety Officer (OEHS) to ensure proper documentation and recordkeeping.

Packaging and Shipping of Infectious Agents Training

OEHS personnel have been trained to package and ship infectious agents such as microorganisms, blood samples, and clinical samples for pathological testing, as required by federal and international regulations. OEHS offers this service free of charge. This does not include transportation cost. Please contact OEHS Director, Lance Franklin, at (57)7-1200 to make arrangements for shipments of hazardous materials or infectious Agents.

Laboratory Specific Training

Individual laboratories are required to develop specific training for the particular agents and procedures that personnel will perform in that laboratory. This training should be specific to the hazards in the laboratory and to each person's laboratory duties. Each person in the laboratory must understand the hazards associated with laboratory operations, how to prevent exposures to biological and chemical agents, and exposure response procedures. Training records, including the names and signatures of the instructor(s) and laboratory personnel, signature of the PI (if not the instructor), topic of training, and date that training was conducted, should be documented and maintained by the laboratory. Ongoing training is required as new hazards and procedures are introduced into the laboratory. The occurrence of spills, spread of contamination, near misses, etc. also indicate the need for refresher training.

Laboratory Safety Training

Laboratory staff working in labs that use hazardous chemicals must receive general Laboratory Safety Training.

Personnel working with radioisotopes or x-ray generating devices must attend radiation safety training. Contact the WSU Radiation Safety Staff at 313-577-1200 for information and scheduling.

Chapter 7

Decontamination

Decontamination of cultures and objects contaminated by biological agents is routinely performed in microbiological laboratories. Decontamination is a vital component of microbiologic al safety practice and serves to protect laboratory personnel (as well as others) from infection, as well as the release of infectious organisms to the outside environment (primarily through person to person transmission). Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of cultured organisms.

Chemical Disinfection

Decontamination of work surfaces, equipment, biological safety cabinets, and other inanimate objects using antimicrobial agents is referred to as disinfection. Several chemical agents are used as disinfectants. Laboratory workers should remember that there are hazards associated with all of these chemical disinfectants. Inhalation and skin contact should be minimized, and eye contact avoided. Appropriate gloves and safety eyewear should always be worn when handling these chemicals. Pertinent information for some of the common chemical disinfectants is summarized in table format at the end of this chapter.

Autoclaving

Autoclaving uses saturated steam under pressure (approximately 15 psi) to achieve a temperature in the autoclave of at least 121^{o}C (250^{o}F). Autoclaving can be used to destroy vegetative bacteria, bacterial spores, and viruses. When decontaminating biohazardous waste, it is recommended that the temperature in the waste reach a minimum of 115^{o}C for a minimum of 20 minutes. The total processing time required to meet these conditions depends on several loading factors (see below); however, it is recommended that a minimum autoclave cycle of one hour be used when decontaminating waste.

There are three factors that in combination determine the effectiveness of autoclaving:

Temperature - autoclave uses steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121^{o}C. Although the autoclave chamber may reach 121^{o}C, this does not necessarily mean that the interior of the load will reach this temperature.

Time - a minimum autoclave cycle time of twenty minutes at a chamber temperature of 121^{o}C (time does not begin as soon as the autoclave cycle is initiated) is commonly recommended for sterilization of clean items. However, the total processing time required to achieve decontamination

depends on several loading factors, including the load container (heat transfer properties), the amount of water added to the load, and the weight of the load. For increased loads, an increased cycle time will be required to ensure effective decontamination.

Contact - steam saturation is essential for maximum heat transfer. Steam must contact all areas of the load. Autoclave bags and other containers should be left partially open (or otherwise permit entry of steam) to ensure adequate contact. Studies have shown that adding water to the interior of the bag improves the time-temperature profile of the autoclave cycle, increasing the sterilization efficiency of the autoclave.

There are specific requirements for decontaminating biohazardous waste prior to disposal. See Chapter 11 for autoclave procedures relating to biohazardous waste.

Dry Heat

Dry heat is less effective than moist heat (autoclaving); requiring higher temperature and longer contact time. Nevertheless, dry heat is preferable to moist heat for decontamination of anhydrous materials and closed containers. This is due to the fact that the moisture component of the steam used in an autoclave will not effectively penetrate anhydrous materials and closed containers. The highest dry heat equivalent temperature that these materials will reach in an autoclave is 121^{o}C. The highest temperature that material will reach in a dry-heat oven will be the actual temperature inside the oven. A temperature of 160-180^{o}C for 3-4 hours is recommended for decontamination of waste using a dry heat oven.

Disinfectant	Use Parameters	Effective Agent[a]					Important Characteristics	Potential Application
		Vegetative Cells	Lipophilic viruses	Tubercle Bacilli	Hydrophillic viruses	Bacterial spores		
Alcohol (ethyl, isopropyl)	Conc: 70-80% *Contact time:* 10-30 min.	+	+	+	±		Eye irritant, toxic, flammable, inactivated by organic matter	Surfaces – work & equipment
Chlorine Compounds	Conc: 0.05 -0.5% (commercial bleach .5%) *Contact time:* 10-3- min	+	+	+	+	±	May leave residue; corrosive; skin, eye & respiratory irritant; inactivated by organic matter; makeup at least weekly	Spills, equipment surfaces, instruments glassware, water baths
Quaternary Ammonium Compounds	Conc: 0.1-2% *Contact time:* 10-30 min	+	+				Toxic, inactivated by organic matter	Surfaces (work & equip.), BSCs, floor maintenances, glassware, instruments
Phenolic Compounds	Conc: 0.2-3% *Contact time:* 10 -30 min	+	+	+	±		Leaves residue; corrosive, skin, eye & respiratory irritant; toxic; inactivated by organic matter	Surfaces (work & equip.), BSCs, floor maintenances, glassware, instruments, water bath
Iodophor Compounds	Conc: 0.47% *Contact time:* 10-30 min	+	+	+	±		Leave residue; corrosive, skin & eye irritant; toxic; inactivated by organic matter	Surfaces (work & Equip.), BSCs, glassware, water baths
Formaldehyde[b] (Formalin)	Conc: 4-8% *Contact time:* 10-30 min	+	+	+	+	±	Leave residue; skin, eye & respiratory irritant; toxic (**carcinogen**)	Less effective than other disinfectants but can be used for equipment surfaces, glassware, instruments
Glutaraldehyde	Conc: 2% *Contact time:* 10-600 min	+	+	+	+	+	Leaves residue; skin, eye & respiratory irritant; toxic	Equipment surfaces, glassware, instruments

From: Laboratory Safety: Principles and Practices, 2nd Edition, Diane O. Fleming, John H. Richardson, Jerry J. Tullis, and Donald Vesley, eds., American Society for Microbiology, Washington, D.C.

a: + (very positive response); ± (less positive response). Blank denotes a negative response or not applicable.
b: due to its irritating characteristics and status as a carcinogen, formaldehyde should only be used with good local exhaust ventilation.0

Chapter 8

Laboratory Ventilation for Biosafety

Chemical Fume Hoods

Traditional laboratory chemical (or fume) hoods are designed to capture and control chemical vapors and pull them away from the worker. Although the inward flow of air provides protection to the user, chemical hoods do not provide protection for the product (the desired organism being manipulated). Unless a High Efficiency Particulate Air (HEPA) filter is added, chemical hoods do not provide protection against release of viable organisms to the environment. The airflow within a chemical hood is often somewhat turbulent, which can potentially result in exposure of the user to the organisms being used. In short, a chemical hood is not a biological safety cabinet, and generally does not provide product protection or environmental protection.

Horizontal Laminar Flow Clean Bench

With horizontal laminar flow clean benches, HEPA filtered air flows horizontally across the workspace directly toward the user. These clean benches provide product protection and were originally designed to provide a particulate free environment for manufacture of semiconductor components. Clean benches do provide product protection against microbial contamination, but they do not provide personal protection or environmental protection. In fact, the horizontal flow of air will blow biological agents directly toward the user and into the laboratory. Clean benches are not a biological safety cabinet, and they should not be used with any materials (biological, chemical, or radiological) requiring containment for protection of personnel or the environment. Clean benches are acceptable for tissue culture work only with cell lines considered to represent low risk (BSL1 agents) to laboratory workers (including immunocompromised individuals who may frequent the lab). Human cell lines and nonhuman primate cell lines are generally considered to be BSL2 agents and would not be suitable for use in a clean bench.

Biological Safety Cabinets

There are three classes of biological safety cabinets (BSCs), class I, II, and III (see schematic below). Class II BSCs are subdivided into type A and type B cabinets. All BSCs provide personnel and environmental protection, with Class II BSCs also providing product protection. Personnel protection is achieved by inward airflow through the front of the cabinet; product protection is achieved by downward HEPA filtered airflow from the top of the cabinet; and environmental protection is achieved by HEPA filtration of exhaust air..

Classes and Types of Biosafety Cabinets

<u>Class I BSCs</u>

Class I BSCs are similar to chemical hoods in that inflow air enters the front of that cabinet, flows across the work area, exits at the rear of the cabinet, and is exhausted outdoors. The primary difference is that chemical hoods usually do not have any

filtration mechanism to prevent contaminants from being released to the outside (unless a filter or scrubber is added), whereas all air exhausted from a Class I BSC must pass through a HEPA filter before being exhausted outdoors. The inflow of air into a Class I BSC provides personnel protection, and HEPA filtration of the exhaust air provides environmental protection; however, Class I BSCs do not provide product protection. Class I BSCs are suitable for work involving BSL 1, 2, or 3 agents when product protection is not required.

Class II Type A BSCs

Type A cabinets have a minimum airflow of 75 feet per minute (fpm), and recirculate approximately 70% of the air as HEPA filtered down-flow air. Some Type A cabinets have potentially contaminated air plenums that are under positive pressure. Any breach of the positively pressured plenum or ducting would result in loss of containment and possible release of material. Although all air is HEPA filtered before it is exhausted, Type A cabinets can be exhausted directly into the room. Type A cabinets are suitable for BSL 1, 2, or 3 agents. Recirculated of air within the cabinet and discharge of exhaust air directly into the room preclude the use of Type A cabinets for volatile chemicals or volatile radionuclides.

Class II Type B BSCs

All Type B cabinets differ from Type A cabinets in three important design features: 1) all potentially contaminated plenums are under negative pressure, 2) exhaust air is discharged directly to the outside rather than to the room, and 3) they have a higher minimum inflow velocity of 100 fpm. Type B3 BSCs Type B3 cabinets are a modified Type A BSC that has no plenums under positive-pressure, and which is exhausted directly to the outside.

Type B3 cabinets are similar to Type A cabinets in that approximately 70% of air is recirculated as HEPA filtered down-flow air. Type B3 cabinets are suitable for BSL 1, 2, or 3 agents and minute quantities of volatile toxic chemicals or tracer amounts of volatile radionuclides..

Type B1 cabinets are designed such that small quantities of carcinogens and volatile radionuclides required for microbiological work can be handled safely. To prevent buildup of these chemicals within the cabinet, down-flow air is “split”, with a portion directed to the front of the cabinet and a portion directed to the back of the cabinet where it is exhausted directly to the outside without recirculation. Volatile chemicals should be handled in the direct exhaust (rear) portion of the cabinet to prevent recirculation. Approximately 30% of outgoing air is recirculated as HEPA filtered, down-flow air. Type B1 cabinets are suitable for BSL 1, 2, or 3 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies if the work is performed in the direct exhaust (rear) portion of the BSC.

Type B2 BSCs

These cabinets are referred to as “total exhaust cabinets” because all inflow and down-flow air passes through the cabinet only once (without any recirculation), and then is

directly exhausted to the outside. Since there is no recirculation of air within the cabinet, down-flow air must be drawn in from the room (at the top of the cabinet) and then HEPA filtered prior to entering the cabinet. Type
B2 cabinets are suitable for BSL 1, 2, or 3 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies.

Class III BSCs

Class III BSCs are of a glove-box design (gas-tight absolute containment) that provides the highest level of personnel protection, as well as product and environmental protection. Both supply and exhaust air are HEPA filtered. These cabinets should be maintained under a minimum negative pressure of 0.5" w.g. Exhaust air is discharged to the outdoors through double HEPA filters (or HEPA and incineration). Class III cabinets provide the highest level of containment and can be used for work involving any infectious agent; however, they are most appropriate for work involving BSL 4 agents.

Certification of BSCs

Generally, commercial BSCs are tested by the cabinet manufacturer in accordance with National Sanitation Foundation (NSF) criteria. Cabinets that meet the NSF criteria for performance characteristics including biological containment, ventilation, cabinet leakage, and HEPA filter leakage are NSF certified. Field certification of BSCs is also required to ensure that the cabinet still performs as it did when it obtained NSF certification at the factory. Field certification is required by the National Institutes of Health (NIH) under the following circumstances: 1) upon installation of a new BSC, 2) annually thereafter, 3) after repair or maintenance is performed, and 4) after the BSC is relocated.

NSF standard 49 provides criteria for construction of BSCs, testing by manufacturers (including biological containment testing), and field certification. NSF has also established a certification program for field certifiers to ensure a minimum level of competency and professionalism. It is recommended that NSF field certifiers be used for field certification of BSCs. Field certification tests include

1. Primary Tests (BSC performance):
    a. Inflow test
    b. Downflow test
    c. Smoke pattern test
    d. HEPA filter leakage
    e. Cabinet leakage (when BSC is newly installed, relocated, or maintenance has been performed that involved removal of access panels)

2. Additional tests (worker comfort and safety): performed at discretion of certifier
    a. Noise
    b. Vibration
    c. Lighting
    d. Electrical leakage, polarity, and ground circuit resistance

Guidelines for Use of Biological Safety Cabinets

The installation and use of a BSC is an indication that safe work practices are needed to prevent contamination and infection. Modern BSCs are extensively engineered and provide excellent containment of microorganisms; however, they are not substitutes for good work practices and can only serve to complement a safe worker. The following are general recommendations for BSC use.

1. Ready Work Area
    a. Turn off UV lamp (if equipped); turn on fluorescent light.
    b. Check air grilles for obstructions; turn on fan (blower).
    c. Allow air to purge workspace for 3 minutes.

2. Pre-disinfect
    a. Spray or swab all interior surfaces with an appropriate disinfectant.
    b. Allow the surfaces to air dry.

3. Assemble Materials
    a. Only introduce materials that are required to perform the procedure.
    b. Position materials so that clean and contaminated items do not touch, with contaminated items downstream (ventilation-wise) of clean items.
    c. Ensure the view screen is properly located and secured.

4. Pre-Purge Cabinet
    a. Allow the BSC fan to run for at least three minutes with no activity inside (leave fan on!).

5. Prepare Self
    a. Don protective clothing, gloves, mask, etc., as appropriate.

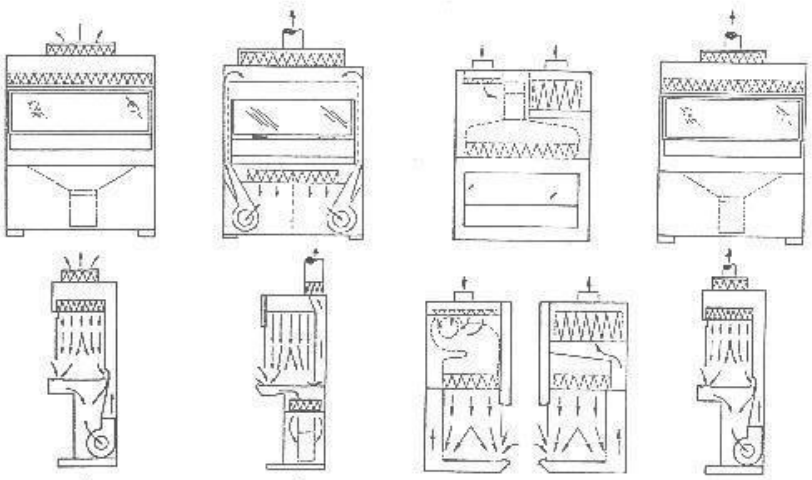
6. Perform Procedures
    a. Minimize movement of arms during procedure; move arms straight in or out of the BSC when entering or exiting.
    b. Work from a clean area to more contaminated work areas (see figure below).
    c. Remove gloves into contaminated material container.

7. Cleanup and Post-disinfection
    a. Place potentially contaminated materials in a biohazard bag or other appropriate container.
    b. Wipe surfaces of all items in the BSC with an appropriate disinfectant.
    c. Remove all items from the BSC and autoclave (or otherwise disinfect) waste and other contaminated materials as appropriate.
    d. Disinfect all surfaces of the BSC.

8. Personal Hygiene
    a. Remove protective clothing, mask, etc., and dispose of as appropriate.
    b. Wash hands.

9. Post-Purge Cabinet
    a. Allow air purge period (minimum of three minutes) with no activity inside (leave fan on!).

10. Shutdown cabinet
    a. Turn off blower and fluorescent lamp.
    b. Turn on UV lamp (if equipped).



Class	II	II	II	II
Type	A	B1	B2	B3
Inflow (fpm)	75	100	100	100
Downflow (fpm)	75	50	50	75
Exhaust (cfm)	45	65	65	65
% Recirculation	70	30	0	70
Use: Biohazards	+	+	+	+
Toxics, Volatiles	-	+	+	±
Protection User, Product	U/P	U/P	U/P	U/P

FIG. 7. Variations in class II cabinets. U, User; P, product.

Kruse, R.H. et al., Biological Safety Cabinetry, Clinical Microbiology Reviews, April 1991, p219

Chapter 9

Human Tissue and Cell Culture

Working with Human Blood or Tissues
All human blood, blood products, body fluids, and tissue are to be assumed to be infectious (the concept of “Universal Precautions”) and must be handled using Biosafety Level 2 (BSL2) practices and procedures. Persons who are exposed to these materials in the laboratory or other occupational setting are considered to have potential exposure to bloodborne pathogens such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV). People exposed to these materials must be included in the WSU Bloodborne Program and complete annual Bloodborne Pathogen Training.

Transmissible spongiform encephalopathies
Spongiform encephalopathies (Creutzfeldt-Jakob, Kuru, and related agents) are fatal prion diseases that have been demonstrated in the brain and spinal cord of infected persons. These agents are resistant to conventional inactivation procedures including chemicals (formalin, alcohol), boiling, dry heat, and irradiation, and these agents can be present in fixed tissue from infected persons. Although nerve tissue (brain, spinal cord) is usually more infectious, all tissues from humans and animals infected with these agents should be considered potentially hazardous. Although laboratory-associated infections have not been demonstrated, it is prudent to consider nerve tissue (even fixed tissue) to be potentially infectious. BSL2 containment and practices are recommended for all activities utilizing known or potentially infectious tissues and fluids from naturally infected humans and from experimentally infected animals. Cell Culture Human or animal pathogens may be associated with cell or organ cultures. Cell cultures known (or suspected) to contain a etiologic agent or an oncogenic virus are classified at the same biosafety level as that recommended for the agent. The following cell cultures and tissues require BSL2 or higher containment and procedures:

1. All cultured cells derived from human sources, including immortalized and “well established” cell lines.
2. All cultured cells derived from primate lymphoid or tumor tissue.
3. All cultured cells exposed to or transformed by a primate oncogenic virus.
4. All clinical materials, such as samples of human tissue obtained from surgery, biopsy, or autopsy.
5. All primate tissue.
6. All uncharacterized cultured cells new to the laboratory until proven to be free of infectious agents.
7. All virus-containing primate cultured cells.
8. All mycoplasma containing cultured cells.

Note: Using cells of human origin invokes the Bloodborne Pathogens Standard and its specific training and work requirements.

Chapter 10

Biohazardous Spill Response

Preplanning for Biohazardous Spill Cleanup

All spills of biohazardous materials do not represent the same risk to personnel and the environment, making each spill somewhat unique. Nevertheless, preplanning of spill response will lower the risk of cleaning up a spill and will increase the likelihood that the spill is handled appropriately. Laboratory supervisors should prepare their laboratory for typical spill scenarios expected in the laboratory. Laboratory workers should be informed of the hazards of the biological agents used in the laboratory, the risk associated with these agents during spill scenarios, how to safely cleanup the agents, and properly dispose of cleanup materials. Each laboratory area should have spill cleanup materials available to respond to the largest spill anticipated for that area. It is recommended that as a minimum, the following spill cleanup materials be available in the laboratory:

- Gloves - thick chemical resistant gloves or double pair of thin, nitrile gloves recommended
- Safety Goggles - face shield is strongly recommended to avoid splashes to the nose and mouth
- Lab coat or smock to protect clothing and body
- Absorbent pads
- Disinfectant appropriate for the agents used in the laboratory
- Forceps or other devices to pick up contaminated material (especially sharps)
- Sharps disposal container
- Autoclavable biohazard bags

Biohazardous Spill Cleanup Procedures

Several factors that must be considered when assessing the risk that a spill presents, including:

- Volume and concentration of the spilled material
- The infectious dose of the spilled material and routes of exposure
- Location of the spill
- Degree of aerosolization of the agent resulting from the spill
- Susceptibility of the spilled material to disinfection
- Nature of the affected surface(s) and its ability to “hide” organisms from disinfection
- Immune status of immediate personnel

As with any spill scenario (biological, chemical, or radiological) the safety of personnel is the most important consideration. Cleanup is to begin only after it is determined that the personnel who will clean-up the spill have appropriate knowledge, training, and equipment.

The following are general biohazardous spill cleanup procedures that are appropriate for most spill scenarios; however, the appropriate response to any spill is based on an assessment of the risk associated with that particular situation.

Biohazardous Spills Inside Biological Safety Cabinets

- Wear laboratory coat (disposable recommended), safety glasses, and gloves (appropriate for the biological agent and the chemical disinfectant) during cleanup.
- Allow the biological safety cabinet to run continually during cleanup.
- Surround the affected spill area with absorbent material to prevent spread of the spill.
- Apply disinfectant appropriate for the biological agent, and allow a minimum of 20 minutes contact time (or as directed by manufacturer's instructions). Alcohol or other flammable liquids are not recommended.
- Wipe up spill with disposable cloth or towel soaked with disinfectant.
- Wipe the walls and work surface of the BSC, and any equipment in the cabinet with a disinfectant-soaked cloth.
- Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
- Allow non-autoclavable items to have a minimum of 20 minutes contact time with disinfectant (or as directed by manufacturer's instructions) before removing from the BSC.
- Remove protective clothing and place in a biohazard waste bag for autoclaving.
- Thoroughly wash hands, forearms, and face with soap and water.
- Allow BSC to run for a minimum of 10 minutes before resuming work in the cabinet or shutting the cabinet off.

Biohazardous Spills in the Laboratory, Outside the Biological Safety Cabinet

- If a BSL1 agent (or less than 100 ml of a BSL2 agent) is spilled, go to step 4.
- If the spill involves greater than 100 ml of a BSL2 agent, immediately evacuate all personnel from the affected area. Wait for aerosol to settle (usually a minimum of 30 minutes) before entering the spill area. Exception: If the laboratory is not under negative pressure, cleanup should begin as soon as possible to minimize the spread of aerosols.
- Notify OEHS at 577-1200 as soon as possible for assistance with the cleanup.
- Remove any contaminated clothing and place in a biohazard waste bag for autoclaving. Wash all affected skin with soap and water.
- Wear a long-sleeved gown or lab coat (disposable recommended), shoe covers, safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant).
- Place absorbent pads over the spill (to absorb liquid), then place a second layer of disinfectant- soaked absorbent pads over the spill.

- Pour additional disinfectant around the spill, being careful to minimize aerosolization, and work from the periphery toward the center, ensuring thorough contact of the spill with the disinfectant. Disinfect all items in the spill area.
- Allow a minimum of 20 minutes contact time (or as directed by manufacturer's directions) with the disinfectant.
- Wipe down all equipment, tools, etc. with disinfectant.
- Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
- Remove protective clothing and place in a biohazard waste bag for autoclaving.
- Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.

Biohazardous Spills Inside a Centrifuge

- Clear the area of all personnel and allow aerosol to settle (usually a minimum of 30 minutes) before re-entering the area.
- Wear a laboratory coat (disposable recommended), safety glasses, and gloves during cleanup.
- Transfer the rotor and buckets to a biological safety cabinet for cleanup.
- Using an appropriate disinfectant, thoroughly disinfect the inside of the centrifuge, and the rotor and buckets.
- Discard cleanup materials and protective clothing as biohazardous waste.
- Thoroughly wash hands, forearms, and face with soap and water.

Biohazardous Spills Outside the Laboratory During Transport

- Immediately clear the area of all personnel and secure the area.
- Cleanup should be initiated as soon as possible to prevent spread of aerosol. Attempt cleanup only if appropriate cleanup materials and protective clothing are available.
- Notify OEHS at 577-1200 as soon as possible for assistance with the cleanup.

Since it is impossible to prevent aerosolization when a spill occurs outside of the laboratory, the primary emphasis when transporting biological agents is on spill prevention. All biological agents are to be transported from the laboratory inside an unbreakable, well-sealed, primary container containing absorbent material that is contained inside of a second unbreakable, well-sealed, secondary container. Both the primary and secondary containers must be labeled with the universal biohazard symbol and the identity of the agent.

Chapter 11

Biohazardous Waste

Biohazardous waste includes waste materials derived from cultures and stocks of infectious agents, human pathological wastes, contaminated animal carcasses and body parts, all sharps, and human blood and blood products. Proper handling and disposal of biohazardous waste is necessary to prevent infection of personnel (laboratory workers, custodians, laboratory visitors, etc.) and release to the environment. OSHA and State regulations require that biohazardous waste be properly labeled, stored, and disposed. In some instances, improper disposal of biohazardous waste has resulted in regulatory action and negative media attention. Thus, the handling and disposal of biohazardous waste has important implications with regard to environmental health and safety, regulatory compliance, legal liability, and public opinion

Labeling and Storage of Biohazardous Waste

Labeling

At a minimum, all biohazardous waste must be labeled with the universal biohazard symbol. Additional information such as the type of waste (such as “sharps”, or “liquid waste”) and origin of the waste is recommended.

Rooms or areas used to store biohazardous waste must be labeled with the following warning, *“CAUTION - INFECTIOUS WASTE STORAGE AREA - UNAUTHORIZED PERSONS KEEP OUT”;* Laboratories where waste is accumulated during experimentation, and where biohazardous waste is not stored, are not considered storage areas and do not have to be labeled as above.

Storage

Laboratories must decontaminate and dispose of laboratory biohazardous waste on a weekly basis.

Handling and Disposal of Biohazardous Waste

Biohazardous wastes are human, animal or plant tissue or fluids that are contaminated with pathogenic organisms.

Biohazards waste bins and a non-autoclavable liner bag are provided to each lab that generates biohazardous waste. The type of waste that is disposed of in red bins includes, but is not limited to:

- Cultures and stocks of etiologic agents
- Human and nonhuman blood, blood products, body fluids, and tissue
- Lab waste contaminated with human or animal blood, blood products, body fluids, or other potentially infectious materials

- Materials and microorganisms used in recombinant DNA technology
- All contaminated glass and plastic ware (pipettes, petri dishes, etc.)

For proper disposal of biological waste, OEHS requires the following:

- Waste should be stored, transported and autoclaved in a proper bag placed into a leak-proof, sealed biohazard Red Bin.
- Indicator tape or steam indicator bags must be used for autoclavable materials.
- After the waste is treated, place it into red bins, tie the liner bag and properly close the lid when the bin is full.

All biohazardous wastes must be clearly marked with the universal biohazard label (see below). If biohazardous waste also contains hazardous or radioactive material, it must be identified as containing both materials; **this type of waste should not be generated if at all possible**. For materials that contain viable organisms and require incineration contact Rob Moon, Biosafety Officer, for disposal instruction at 313-577-1200.

SHARPS

Sharps materials (needles, syringes, scalpels, etc.) must be placed in marked biohazard sharps containers. Biohazardous waste is picked up by OEHS.

Sharps containers are provided free of charge. Place containers close to areas where work with sharps is done, don't allow containers to overfill, and **NEVER RECAP NEEDLES!**

Examples of "SHARPS" include, but are not limited to:

- Needles and syringes
- Scalpels and lancets
- Razor and microtome blades
- IV tubing with needles attached
- Contaminated glass, Pasteur pipettes
- Any other sharp, metal lab waste that could puncture a bag

UNIVERSAL BIOHAZARD SYMBOL

Uncontaminated Glass, Broken Glass, and Plastic Waste

Laboratory glass and plasticware that is not contaminated with human or animal blood or other potentially infectious biological material may be disposed of in sturdy cardboard boxes. Boxes should not weigh more than about 25 lbs. when full. Tape boxes shut and label them “broken glass” for collection by custodial staff.

Human Pathological Waste

Pathological waste includes all recognizable human anatomical remains (teeth should be disposed of as sharps). Collect human pathological waste in a red, biohazard bag (or other appropriate, labeled container) and place inside a rigid container that can be sealed and which is labeled with the universal biohazard symbol. Contact the Body Bequest for more information concerning the disposal of recognizable human anatomical remains.

Chapter 12

Transfer and Receipt of Select Agents

The Centers for Disease Control and Prevention (CDC) require registration of facilities that ship or receive infectious agents and toxins that are included on the CDC's list of Select Agents (see end of this Chapter). Failure to register with the CDC will preclude transfer or receipt of these agents. Individual laboratories must notify the Office of Environmental Health and Safety (OEHS) of their intention to ship or receive any of these agents so that proper CDC notification can be made. These regulations also govern intra-facility transfer of Select Agents, as well as storage and disposal. The CDC is expected to make periodic inspections of WSU laboratories and records to ensure compliance with NIH and OSHA requirements.

Transfer of Select Agents from an off-site supplier to WSU

The WSU Institutional Biosafety Committee (IBC) must approve new use of Select Agents. The PI for each laboratory must submit a description of the research project involving the Select Agent, including estimated amounts to be used, frequency of use, conditions of use, storage conditions (including security), decontamination and spill response, and disposal. The "Biological Agents - User Application Form" form located at the end of Chapter 2 is to be used for these submissions.

1. The IBC will respond to the PI making the request. The IBC can approve the requests is, ask that modifications to the protocol be made, or deny approval of the request.

2. The requesting laboratory must complete the following sections of CDC form EA-101. Complete block 1 in its entirety and only the following portion of block 2: Requestor Name (PI's name), Signature (PI's signature), and Phone/Fax. Then forward the form, a copy of the IBC approval (new requests only), and a copy of the Purchase Order (with PI's name as identifier) to Tom Perez, OEHS, 5425 Woodward, Suite 300 for approval as "Responsible Facility Official" (block 2).

   Note: The requesting laboratory is responsible for ordering the Select Agent from the supplier (who must also be registered with the CDC).

3. OEHS will authorize the EA-101 (by completing block 2.) and return the form and a copy of the WSU Facility Registration Certificate to the requesting laboratory. The laboratory must forward the EA-101 and the Registration Certificate to the supplier of the Select Agent.

4. The supplier must verify that WSU is a registered facility under the CDC Select Agent Transfer Program, and that the requesting individual (the PI) is a WSU employee and authorized user.

5. After verification, the supplier will complete block 3 and block 4 (except for the date received) of the EA-101 and send the Select Agent and the form to WSU. The supplier should also include a copy of their facility's Registration Certificate.

6. The laboratory is to notify OEHS Biosafety Officer as soon as possible after arrival of the Select Agent at WSU. Within 36 hours, OEHS (WSU) is required to notify the supplier that the agent was received at WSU. OEHS must follow up this initial acknowledgement by sending the supplier a paper copy or fax within three business days acknowledging receipt of the Select Agent.

7. Within 24 hours after acknowledgement from the requesting facility (WSU) that the Select Agent was received at the requesting facility, the supplier must complete block 4 of the EA-101 (Date agent received) and forward a copy of the EA-101 to the CDC.

8. The PI must notify OEHS when the Select Agent has been depleted or destroyed.

9. OEHS must then complete block 5 of the EA-101 indicating the date the agent was depleted or destroyed, and forward a copy of the completed EA-101 to the CDC.

Transfer of Select Agents from WSU to another facility
This process is the same as the above procedure with WSU being the supplier, instead of the requesting facility.

Transfer of Select Agents from one WSU laboratory to another
Completion of an EA-101 form is not required but OEHS must be notified prior to the transfer. The name and location of the recipient, the amount transferred, and the date of transfer must be documented. The PI requesting the Select Agent must be included on the WSU Select Agent registration that is on file with the CDC, prior to the transfer. The supplying and receiving laboratories must update their Select Agent inventories. The receiving laboratory must comply with all requirements of the Select Agent program, including storage and disposal.

Addition or deletion of Select Agents that WSU can transfer or receive
OEHS must be notified of any addition or deletion of Select Agents in a particular laboratory. In addition, the CDC must be notified of any addition or deletion of Select Agents that WSU (as an entire facility) intends to transfer or receive.

Addition or deletion of laboratories or Principal Investigators
OEHS must be notified of any addition or deletion of laboratories or PIs transferring or receiving Select Agents; OEHS must then notify the CDC of these changes.

Inventory of Select Agents
An accurate, up to date inventory of all Select Agents must be maintained. For toxins this will require that a usage log be kept that includes the date of use, amount used, and date and method of inactivation. For biological organisms, a usage log must be maintained that includes the date of use, and the date and method of destruction.

Storage of Select Agents
Select Agents must be stored so as to minimize exposure to personnel, as well as prevent leaks, spills or other release of the agent. Security must be maintained to prevent unauthorized access to Select Agents.

Disposal of Select Agents
The depletion or destruction of all Select Agents must be carried out on-site using recognized decontamination methods such as chemical treatment or autoclaving – dilution by flushing down the sink is not permitted. These inactivation requirements apply to all Select Agents at WSU, including those that were acquired prior to the formal CDC requirements outlined here. OEHS must be notified of final depletion or destruction (all of the agent has been used or destroyed) so that this information can be forwarded to the CDC.

Availability of EA-101 Forms
Access to EA-101 forms (blank or completed) is limited to PIs whose laboratories are registered with the CDC and those personnel designated by the PI. These forms must be stored so that they are not readily available to casuals who may enter the laboratory. Blank EA-101 forms are available from OEHS.

More information on Select Agents