
BIOSAFETY MANUAL

Belongs To:
Wake Forest University Health Sciences
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BIOSAFETY PROCESS MANUAL**Wake Forest University School of Medicine****Table of Contents**

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BIOSAFETY PROCESS MANUAL

CHAPTER 1- INTRODUCTION

Policy

The Biosafety Process provides a method for the establishment and maintenance of a system for the control of biohazards in academic and research programs. It is the responsibility of all faculty, staff, and students to file Biosafety Applications or Registrations for designated activities and to follow the practices required for the use of biohazardous materials.

Purpose

This document provides the basic outline and policy and procedures for the Wake Forest University and Wake Forest University Health Sciences Biosafety Program. A number and a variety of potentially hazardous biological materials are required in the teaching and research programs of The University and The School of Medicine. The Biosafety Policy is designed to:

- Ensure that the Wake Forest University and Wake Forest University Health Sciences complies with the Guidelines of National Institutes of Health and other research sponsors, and all applicable federal, state, and other regulations respecting biohazardous material management.
- Protect Wake Forest University and Wake Forest University Health Sciences faculty, staff, students and the general public from laboratory acquired infections and to control the spread of contamination as the result of activities in which biohazardous materials are used.

Scope

This policy applies to all Wake Forest University and Wake Forest University Health Sciences campuses, faculty members, basic scientists, supervisors, and employees.

The Biosafety Process shall apply to the use of potentially oncogenic, infectious or toxic biological materials in all The University and The School of Medicine teaching programs and in all research projects. In addition, the policy shall apply to research involving the use of Wake Forest University and Wake Forest University Health Sciences facilities and to research conducted by private organizations involving the use of Wake Forest University and Wake Forest University Health Sciences facilities under an agreement with the Wake Forest University and Wake Forest University Health Sciences.

Authority

CDC 93-8395	Biosafety in Microbiological and Biomedical Laboratories
NIH 97-3	Guidelines for Research Involving Recombinant DNA Molecules (National Institutes of Health Guidelines)
OSHA 29 CFR 1910.1030	Bloodborne Pathogens
OSHA 29 CFR 1910.1450	Occupational exposure to hazardous chemicals in laboratories.

CHAPTER 2 - ROLES AND RESPONSIBILITIES

Biosafety Committee

The Wake Forest University and Wake Forest University Health Sciences Biosafety Committee, which has representation from Wake Forest University Health Sciences, Wake Forest University, Employee Health Services, Wake Forest University Health Sciences Environmental Health & Safety Program, has responsibility for the establishment and maintenance of a system for the control of biohazards in academic and research programs. This Committee performs the following functions:

- Develop a comprehensive Health and Safety Program for all academic work deemed to involve a biohazard.
- Authorized to identify and approve for biohazard safety all research and teaching activities involving biohazardous materials. Approval involves the assessment of the principal investigator's qualifications and experience relative to biohazardous materials, the level of containment required and the containment facilities available, and work procedures for storage, handling, and manipulation of biohazardous agents. This assessment may require an inspection of the facility and the preparation of a document outlining specific recommendations for the management of biohazardous materials and the health surveillance of exposed personnel.
- Provide the WFUHS Office of Research with any certifications required for research grant and contract applications.
- Review periodically the Biohazards Safety Policy and develop appropriate procedures.
- Reviews the effectiveness of the Biological Safety Cabinet Inspection Programs.
- Incorporate initial inspection results into considerations regarding Biosafety Applications.
- Review results of inspection program and make recommendations for improvement.
- Recommend disciplinary action to the Associate Dean for Research and Technology.

WFUHS Environmental Health and Safety

- Maintain a database of all research being conducted under the Biosafety Program.
- Maintain a current list of all locations where biohazardous research is being conducted.
- Conduct regular inspections of all locations where biohazardous research is being conducted.
- Maintain a current inventory of biological safety cabinets.
- Provide staff support for Biosafety Committee activities.
- Maintain this document.
- Conduct periodic surveillance of locations where biohazardous research is being conducted in accordance with research protocols.
- Ensure that faculty and staff who are exposed to biohazards are trained in the handling of these materials, and facilitate the training of such personnel.

- Advise the Office of Research on other matters relating to biohazard safety.
- Inspect laboratories where recombinant DNA, microbiological and biomedical research or chemical usage.
- Maintain central file of biological safety cabinet certifications.
- Approve vendors for biological safety cabinet maintenance.

Principal Investigators

General

- Be adequately trained in good microbiological techniques.
- Provide adequate facilities, equipment and procedures in accordance with established biosafety guidelines and requirements.
- Adhere to Biosafety Committee-approved emergency plans for handling accidental spills and personnel contamination.
- Arrange for maintenance and certification of biological safety cabinets as required by this document.
- Abide by conditions of the Biosafety Application and/or Biosafety registration.
- Allow inspection of laboratory by EH&S.

Hazardous Chemicals

- In accordance with the Chemical Hygiene Plan, submit all protocols involving the use of select carcinogens, reproductive toxins and highly toxic chemicals to the Chemical Safety Committee.

Infectious Agents (Bacteria, Fungi, Viruses, and other Parasites) Biosafety Level 1

- Adhere to requirements of Chapter 5 and referenced documents
- #### Human blood, body fluids
- Adhere to requirements of Chapter 6 and referenced documents

Working with Human and Other Primate Cells and Tissues

- Adhere to requirements of Chapter 7 and referenced documents

Exempt Recombinant DNA

- Adhere to the requirements of Chapter 8 and referenced documents.
- Submit Biosafety Registration (reference [Appendix D2](#)) to Environmental Health and Safety.

Non-Exempt Recombinant DNA

- The principal investigator is responsible for full compliance with the National Institutes of Health Guidelines in the conduct of recombinant DNA research.

General Responsibilities

- Not to initiate or modify recombinant DNA research which requires Biosafety Committee approval prior to initiation (see Experiments Covered by the National Institutes of Health Guidelines) until that research or the proposed modification has been approved by the Biosafety Committee and has met all other requirements of the National Institutes of Health Guidelines;
- Determine whether experiments are covered by Section III-D of the National Institutes of Health Guidelines, Experiments that Require Biosafety Committee Notice Simultaneous with Initiation, and that the appropriate procedures are followed;
- Report any significant problems, violations of the National Institutes of Health Guidelines, or any significant research-related accidents and illnesses to the Biological Safety Officer, Animal Resource Program Director, Biosafety Committee, and /or the National Institutes of Health/Office of Recombinant DNA Activities, and other appropriate authorities (if applicable) within 30 days (reports to National Institutes of Health /Office of Recombinant DNA Activities shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838;
- Report any new information bearing on the National Institutes of Health Guidelines to the Biosafety Committee and to National Institutes of Health/Office of Recombinant DNA Activities (reports to National Institutes of Health/Office of Recombinant DNA Activities shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838);
- Comply with shipping requirements for recombinant DNA molecules;
- Follow the requirements of Section IV-B-4-b of National Institutes of Health Guidelines with regard to submissions by the principal investigator to the National Institutes of Health/Office of Recombinant DNA Activities;
- Follow the requirements of Section IV-B-4-c of National Institutes of Health Guidelines with regard to submissions by the principal investigator to the Biosafety Committee;
- Follow the requirements of Section IV-B-4-d of National Institutes of Health Guidelines with regard to the responsibilities of the principal investigator prior to initiating research; and
- Follow the requirements of Section IV-B-4-e of the National Institutes of Health Guidelines (97-3) with regard to the responsibilities of the principal investigator during the conduct of the research.

Infectious Agents (Bacteria, Fungi, Viruses, and other Parasites) Biosafety Level 2 and above

- Adhere to requirements of Chapter 9 and referenced documents

Human Gene Therapy

- Adhere to requirements of Chapter 10 and referenced documents

Employee Health Services

- The Medical Director of Employee Health Services will be a member of the Biosafety Committee.

- Conduct medical surveillance of employees and students deemed subject to exposure to biohazardous agents by the Committee. Medical surveillance will include, at the discretion of the Medical Director of Employee Health Services, medical examinations, immunizations, and/or Medical Monitoring Programs.
- Maintain necessary records including data on those biohazardous agents to which personnel under medical surveillance may be exposed.

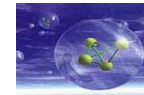
Other Investigators, Employees, and Students

- Attend all necessary training sessions.
- Follow the approved protocols for the lab in which they are working.

CHAPTER 3 - BIOSAFETY PROTOCOL APPROVAL PROCESS



Biosafety Protocol Approval Process



Research Involving	Biosafety Level	Reference or Guidance Information	Submission Document	Approval Source
<i>Bacteria Fungi Parasites</i>	1	<i>Bacteria, fungi, or parasites which are not included on the list of BSL 2 and 3 agents</i>	<i>Biosafety Registration Form</i>	<i>WFUSM EH & S</i>
<i>Human blood Body Fluids OPIM</i>	2	<i>WFUSM Bloodborne Pathogens Exposure Control Plan</i>	<i>Biosafety Registration Form</i>	<i>WFUSM EH & S</i>
<i>Tissue, organ, or cell cultures of human or other primate origin</i>	2	<i>BMBL Appendix H Working with Human and Other Primate Cells and Tissues</i>	<i>Biosafety Registration Form</i>	<i>WFUSM EH & S</i>
<i>Recombinant DNA</i>	2, 3 and 4	<i>Table of Recombinant DNA Approval Levels</i>	<i>Biosafety Committee Application Form</i>	<i>Institutional Biosafety Committee</i>
<i>Bacteria, Viruses Fungi, and Parasites</i>	2, 3 and 4	<i>List of BSL 2 and 3 Agents List of BSL 4 Agents</i>	<i>Biosafety Committee Application Form</i>	<i>Institutional Biosafety Committee</i>
<i>Human Gene Therapy</i>	All levels	<i>Biosafety Program Guidelines</i>	<i>Human Gene Therapy Application Form</i>	<i>Institutional Biosafety Committee</i>

CHAPTER 4 - PROJECTS INVOLVING CHEMICAL AGENTS WHICH ARE SELECT CARCINOGENS, REPRODUCTIVE HAZARDS, OR HAVE A HIGH DEGREE OF ACUTE TOXICITY

Projects that include the use of carcinogenic, mutagenic, reproductive toxins, high acute toxicity chemicals, or select toxins require the approval of the Chemical Safety Committee.

A listing of materials subject to Chemical Safety Committee Approval is found in List of Chemical Substances Subject to Chemical Safety Committee Approval.

CHAPTER 5- INFECTIOUS AGENTS (BACTERIA, FUNGI, VIRUSES, AND OTHER PARASITES) BIOSAFETY LEVEL 1

Other research currently in progress or contemplated at Wake Forest University and Wake Forest University Health Sciences which is exempt recombinant DNA or involves the use of Biosafety Level 1 Microorganisms must be registered through Environmental Health and Safety.

Such experiments must be conducted under Biosafety Level I conditions and must be registered with Environmental Health and Safety at the time the experiments are begun. The Biosafety Registration is included as Appendix D2.

A listing of Biosafety Level 2, 3 and 4 organisms is included in [List of Biosafety Level 2, 3 And 4 Agents](#).

CHAPTER 6 - HUMAN BLOOD, BODY FLUIDS

For research using blood, certain other body fluids, unfixed human tissue, or primary human cell culture, the requirements of the OSHA Bloodborne Pathogens Standard that must be met include the following:

- Training;
- Offer of Hepatitis B vaccine;
- Signing of Informed Consent Form or Declination Form; and
- Compliance with other aspects of the standard and the institutional Bloodborne Pathogen Control Policies and Procedures.
- According to the CDC Guidelines, Biosafety Level 2 conditions are appropriate for such work.

Individuals working with human immunodeficiency virus (HIV), Hepatitis B virus (HBV) or other Bloodborne Pathogens should consult the WFUHS Exposure Control Plan for Bloodborne Pathogens;

- Biosafety Level 2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV-or HBV-infected or inoculated laboratory animals.
- Activities such as the production of research-laboratory scale quantities of HIV or other Bloodborne Pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a Biosafety Level 2 facility using the additional practices and containment equipment recommended for Biosafety Level 3.
- Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a Biosafety Level 3 facility, or Biosafety Level 3 Large Scale if appropriate, using Biosafety Level 3 practices and containment equipment.

Work with Human blood, body fluids require the filling of a Biosafety registration (Reference [Appendix D2](#))

CHAPTER 7 - WORK INVOLVING TISSUES, ORGAN, OR CELLS OF HUMAN OR OTHER PRIMATE ORIGIN

The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV and HIV, as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissues.

Other primate cells and tissues also present risks to laboratory workers. Potential hazards to laboratory workers are presented by cells transformed with viral agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material. Tumorigenic human cells also are potential hazards as a result of self-inoculation.

Additionally, tissues from humans or animals may have proteinaceous infectious particles that lack nucleic acids which are known as prions. A comprehensive listing of prions is found in [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#) 4th Edition

Human and other primate cells must be handled using Biosafety Level 2 practices and containment.

All work should be performed in a biosafety cabinet, and all material should be decontaminated by autoclaving or disinfection before discarding.

Employees working with human cells and tissues should be enrolled in the institutional Bloodborne Pathogens Program, and should work under the policies and guidelines established by the WFUSM Exposure Control Plan.

Employees should provide a baseline serum sample, be offered hepatitis B immunization, and be evaluated by a health care professional following an exposure incident.

Work with these materials requires the submission of a Biosafety Registration (reference [Appendix D2](#)) to Environmental Health and Safety.

CHAPTER 8 - RECOMBINANT DNA APPROVALS

NOTE: Section references refer back to National Institutes of Health Guidelines. A summary of recombinant DNA approval levels is found in [Appendix A - Table of Recombinant DNA Approval Levels](#).

Exempt Recombinant DNA Projects

This includes research in which DNA from an organism is cloned and propagated in *E. coli* or *Saccharomyces cerevisiae*, as long as the *E. coli* strain does not contain a conjugation proficient plasmid or a generalized transducing phage and as long as fermentations do not exceed 10 liter volumes at any one time.

Also included in this category are experiments in which recombinant DNA molecules derived entirely from non-viral sources are propagated in cells in tissue culture.

Experiments in this category must be registered with EH&S and must be conducted at the appropriate biosafety level.

Research work in the exempt category is carried out under Biosafety Level I conditions.

Non-Exempt Recombinant DNA Projects

Experiments in this category must be approved by the Biosafety Committee and must be conducted at the appropriate biosafety level.

Biosafety Level 1 procedures are appropriate for experiments with whole animals if the recombinant DNA vectors used either do not contain viral sequences or contain less than two-thirds of a eucaryotic viral genome.

Biosafety Level 2 procedures are required for work involving viral recombinant DNA vectors. Included in this category are:

Experiments using animal viruses as vectors;

Experiments in which parts of animal or plant viruses are cloned into any procaryotic or lower eucaryotic host other than *E. coli* or *Saccharomyces cerevisiae*;

Experiments involving the use of infectious animal or plant viruses or defective animal or plant viruses in the presence of helper virus in tissue culture systems, and experiments using whole animals; and

Any of these experiments using any of the animal viruses currently employed at Wake Forest University and Wake Forest University Health Sciences must be conducted under Biosafety Level 2 conditions.

CHAPTER 9 - INFECTIOUS AGENTS (BACTERIA, FUNGI, RICKETTSIA, VIRUSES, AND OTHER PARASITES) BIOSAFETY LEVEL 2 AND ABOVE

1. The use of infectious agents that require Biosafety 2 or above facilities and procedures requires the submission of a Biosafety Application to the Biosafety Committee.
2. Requirements for Biosafety Level 2 and 3 facilities are provided in Chapter 13 of this document.

CHAPTER 10 - HUMAN GENE THERAPY TRIALS

Clinical research with genetic material poses many safety and methodological challenges not shared by other forms of human investigation. As a result, there are a number of regulatory requirements that must be satisfied before any human studies involving gene transfer can be initiated. Presented below are guidelines to assist the clinical investigator in opening a gene therapy study at Wake Forest University Health Sciences. WFUHS will confirm that a protocol is approved by all of the necessary review committees before the first patient is enrolled. Investigators may choose to submit a proposal to the National Gene Vector Laboratories (NGVL) for clinical grade vector for use in human trials.

Regulatory Requirements:

The following reviewing bodies must approve clinical protocols that involve the administration of recombinant DNA products into human subjects:

Federal Requirements:

- 1) Food and Drug Administration (FDA) <http://www.fda.gov/cber/>

The pharmaceutical sponsor or the principle investigator must file an Investigational New Drug (IND) application with the Center for Biologics Evaluation and Research (CBER) for each new investigational agent intended for human study. The format and requirements of the IND are provided in Title 21 of the Code of Federal Regulations. The IND must include extensive documentation regarding methods of production and preclinical testing. If an IND has already been filed for the agent, every new protocol must be submitted as an amendment to the IND. Requests for a CBER IND Packet can be made by contacting:

A Office of Communication, Training and Manufacturers Assistance (OCTMA)
HFM-40301-827-1800

The FDA has published a document entitled "Guidance for Human Somatic Cell Therapy and Gene Therapy" <http://www.fda.gov/cber/gdlns/somgene.txt>, which provides guidelines and recommendations relating to the clinical use of gene products.

- 2) NIH-Office of Recombinant DNA Activities (ORDA)/Recombinant DNA Advisory Committee (RAC) <http://www4.od.nih.gov/oba/guidelines.html>. Applications must be in accordance with the "Guidelines for Research involving Recombinant DNA Molecules", Appendix M. This application requires information about the disease under study, vector being used, and nature of the proposal in addition to other items. The NIH review process is open to the public so proprietary information should be withheld. Approval must be obtained from the HIC and IBC before submission to ORDA

Local Requirements:Institutional Biosafety Committee (IBC)

The mission of the IBC is to ensure that biologic agents, including genetic material, are being handled using appropriate safe techniques and that risk of transmission to others is minimal.

Institutional Review Board

The Institutional Review Board (IRB) is charged with review of all research, development, and related activities in which human subjects will participate. This applies to any research involving human subjects conducted by a member of the faculty or staff of the Wake Forest University School of Medicine (WFUSM), whether conducted at the medical school or elsewhere. The Board and each investigator must comply with regulations titled "Protection of Human Subjects" published in the Code of Federal Regulations (45CFR46). This procedure provides evidence that the investigator and the institution have complied with all applicable laws and regulations concerning human research, including review of protection of the rights and welfare of the individuals involved, the appropriateness of methods used to secure informed consent, and evaluation of potential risk to subjects in relation to the potential medical benefits of investigation.

Safety Concerns:

The following questions must be adequately addressed in the body of the protocol otherwise delays in the regulatory process will be inevitable:

Vector information:

- 1) Is a vector being used?
- 2) Source of the vector?
- 3) What type of vector?
- 4) BSL?
- 5) Replication competent?
- 6) Risk of viral shedding? Where (what body fluid) is virus shed?

DNA product administration:

- 1) Where will the DNA product be stored?
- 2) Where will the DNA product be prepared?
- 3) How will the DNA product be transported from the place of storage?
- 3) Where will the DNA product be administered?

Patient Factors:

- 1) Where will the patient be treated?
- 2) Will patients be isolated, what type of isolation, for what duration?
- 3) How and where will patient specimens be obtained, transported, stored?

National Gene Vector Laboratories

The National Gene Vector Laboratories (NGVL) an extramural program of the National Institutes of Health (NIH), are composed of an interactive group of academic production laboratories whose purpose is to provide eligible investigators with clinical grade vectors (DNA plasmid, lentivirus, HSV, adenovirus, adeno associated virus, and retroviral vectors) for phase I and II gene therapy applications.

The Scientific Review Board and Steering Committee of the NGVL will review requests for vector production with selection based upon scientific merit, feasibility and availability of NGVL resources. If the application is approved through the Steering Committee, clinical grade material will be produced free of charge for use in human gene therapy trials.

CHAPTER 11 - ONCOGENIC VIRUSES

The NCI has prepared minimum safety guidelines for research involving oncogenic viruses which are designed to protect the laboratory worker and his/her experiments, to minimize hazards to anyone else who might enter the laboratory area, and to insure the safety of the surrounding community. NCI strongly recommends that the guidelines be practiced in all research laboratories where oncogenic viruses are present.

It is assumed that oncogenic viruses vary in their potential hazard to man. Criteria have been developed to identify oncogenic viruses of moderate and high risk. All other oncogenic viruses are considered low risk. The criteria are not absolute, but are subject to modification as research continues.

In addition to the listed criteria, the extent to which there is prior experience with the virus without indication of any harmful effect on man must also be considered when evaluating risk. Listed below are some known low-risk and moderate-risk oncogenic agents, respectively.

In general, low-risk oncogenic viruses may be handled at Biosafety Level 1 and moderate risk oncogenic viruses may be handled at Biosafety Level 2. All viruses of human or primate origin must be handled at Biosafety Level 2 or higher.

Low Risk Oncogenic Viruses

- Rous sarcoma
- SV-40
- CELO
- Ad7-SV40
- Polyoma
- Bovine papilloma
- Rat mammary tumor
- Avian leukosis
- Murine leukemia
- Murine sarcoma
- Mouse mammary tumor
- Rat leukemia
- Hamster leukemia
- Bovine leukemia
- Dog sarcoma
- Mason-Pfizer monkey virus
- Marek's
- Guinea pig herpes
- Lucke (Frog)
- Adenovirus
- Shope fibroma
- Shope papilloma

Criteria for Moderate Risk Oncogenic Viruses

- A. Suspected oncogenic virus isolate from man.
- B. Virus that transforms human cells in vitro, as evidenced by a morphological and /or functional alteration that is transferred genetically.
- C. Virus that produces cancer without the aid of experimental host modification in either a subhuman primate at any age or across another mammalian in species barrier in juvenile or adult animals.
- D. A genetic recombinant between an animal oncogenic virus and a microorganism infectious for man shall be considered moderate risk until its oncogenic potential for man is determined.
- E. Any concentrated oncogenic virus or infectious transforming viral nucleic acid.

Moderate-Risk Oncogenic Viruses

Ad2-SV40
FeLV
HV Saimiri
EBV
SSV-1
GaLV
HV ateles
Yaba
FeSV

Criteria For High Risk Oncogenic Viruses

A virus proved to induce cancer in man shall be classified as high risk until its complete hazard potential can be determined. At the present time, there are no known oncogenic viruses classified as high risk.

CHAPTER 12 - MEDICAL SURVEILLANCE

Wake Forest University and Wake Forest University Health Sciences requires that researchers whose projects involve the use of infectious/pathogenic agents state in Section 3 Description of Experiments whether or not medical surveillance is necessary.

In Section 13, Description of Monitoring Requirements states exactly what medical surveillance activities are necessary and their frequency.

The committee upon review of the protocol may at its discretion add or delete medical surveillance requirements

CHAPTER 13 - SAFETY AND RISK ASSESSMENT

Risk Assessment

Risk Groups

- Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans.
- Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
- Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.
- Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic intervention is not usually available.

Criteria for Risk Groups

- Classification of agents in Appendix B of National Institutes of Health Guidelines, Classification of Human Etiologic Agents on the Basis of Hazard, is based on the potential effect of a biological agent on a healthy human adult and does not account for instances in which an individual may have increased susceptibility to such agents, e.g., preexisting diseases, medications, compromised immunity, pregnancy, or breast feeding (which may increase exposure of infants to some agents).
- In deciding on the appropriate containment for an experiment, the initial risk assessment from Appendix B of National Institutes of Health Guidelines, Classification of Human Etiologic Agents on the Basis of Hazard should be followed by a thorough consideration of the agent itself and how it is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as:
 - ✓ Virulence;
 - ✓ Pathogenicity;
 - ✓ Infectious dose;
 - ✓ Environmental stability;
 - ✓ Route of spread;
 - ✓ Communicability;
 - ✓ Operations;
 - ✓ Quantity;
 - ✓ Availability of vaccine or treatment; and
 - ✓ Gene product effects such as toxicity, physiological activity, and allergenicity.
- Any strain that is known to be more hazardous than the parent (wild type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain.
- A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment. The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Biosafety Committee must approve the risk

assessment and the Biosafety containment level for recombinant DNA experiments described in Table of Recombinant DNA Approval Levels.

- Experiments that require Biosafety Committee Approval, Recombinant DNA Advisory Committee (RAC) Review, and National Institutes of Health Director approval before initiation;
- Experiments that require National Institutes of Health /Office of Recombinant DNA Activities and Biosafety Committee approval before initiation; and
- Experiments that require Biosafety Committee approval before initiation.
- Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents.
- Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation, and containment features not addressed in Biosafety in Microbiological and Biomedical Laboratories.

Types of Containment

- Physical Containment

Four Biosafety Levels are described in Paragraph J. These Biosafety Levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

- Biological Containment

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers existing that limit either are:

- The infectivity of a vector or vehicle (plasmid or virus) for specific hosts,
- its dissemination and survival in the environment.

Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory containment).

- Combinations

Since these three means of containment are complementary, different levels of containment can be established that applies various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the National Institutes of Health Guidelines.

Physical containment conditions within laboratories may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I of National Institutes of Health Guidelines, biological containment may not be appropriate for all organisms, particularly higher eucaryotic organisms.

CHAPTER 14 - LABORATORY BIOSAFETY LEVEL CRITERIA

The essential elements of the four Biosafety Levels for activities involving infectious microorganisms and laboratory animals are summarized below. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Please refer to [Biosafety in Microbiological and Biomedical Laboratories](#) for standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 1.

Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that:

Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists;

Access to the laboratory is limited when work is being conducted;

Extreme precautions are taken with contaminated sharp items; and

Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Please refer to [Biosafety in Microbiological and Biomedical Laboratories](#) for standard and special practices, safety equipment, and facilities that apply to agents assigned to Biosafety Level 2.

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g. access zone, sealed penetrations, directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g. diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in Biosafety Level 2 facilities. However, the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 must be rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

Please refer to [Biosafety in Microbiological and Biomedical Laboratories](#) for standard and special practices, equipment, and facilities that apply to agents assigned to Biosafety Level 3.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents, which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease.

Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents, and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics.

They are supervised by competent scientists who are trained and experienced in working with these agents.

The laboratory director strictly controls access to the laboratory. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted.

Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

Please refer to [Biosafety in Microbiological and Biomedical Laboratories](#) for standard and special practices, equipment, and facilities that apply to agents assigned to Biosafety Level 4.

CHAPTER 15 BIOLOGICAL SAFETY CABINETS

It is the policy of Wake Forest University Health Sciences that biological safety cabinets shall be tested and recertified annually. The interval for biological safety cabinets used for cytotoxic drugs shall be semiannual. Biosafety cabinets will be decontaminated prior to any move.

This requirement applies to both research and clinical laboratories where biological safety cabinets are used

Features of Biological Safety Cabinets

Biological Safety Cabinets have different features, which affect their use. Table 1 is a comparison of Biosafety Cabinet characteristics.

Table 1. Comparison of Biosafety Cabinet Characteristics

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front; exhausted through HEPA to the outside or into the room through HEPA 2)	YES	YES (1)
II, A	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a thimble unit	YES	NO
II, B1	100	Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	YES	YES (minute amounts (2))
II, B2	100	No recirculation; total exhaust to the outside through hard-duct and a HEPA filter	YES	YES (small amounts)
II, B3	100	Same as II, A, but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter	YES	YES (minute amounts (2))
III	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series	YES	YES (small amounts)

(1) Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I cabinet in to a room should not occur if volatile chemicals are used.

(2) In no circumstances should the chemical concentration approach the lower explosion limits of the compound.

Certification and Maintenance Frequency

The following intervals are established for certification and maintenance of Biological safety cabinets.

Cancer Drugs

- Every six months

Recombinant DNA, Microbiological and Biomedical Laboratories

- Annual

Certification Requirements and Maintenance Data Base

The operational integrity of a new BSC must be validated by certification before it is put into service or after a cabinet has been repaired or relocated. Relocating a BSC may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC shall be tested and certified at least annually to ensure continued proper operation.

Experienced, qualified personnel must perform on-site testing following the recommendations for field testing (NSF Standard 49).

Required tests are listed in Table 2.

Table 2. Performance Tests to be Applied to the Three Classes of Biological Safety Cabinets

Tests Performed For Biosafety Cabinet			
	Class I	Class II	Class III
PRIMARY CONTAINMENT			
Cabinet Integrity	N/A	A	A
HEPA Filter Leak	Req.	Req.	Req.
Downflow Velocity Profile	A	Req.	Req.
Negative Pressure/Ventilation Rate	B	A	Req.
Airflow Smoke Patterns	Req.	Req.	E/F
Alarms and Interlocks	C, D	C, D	Req.
ELECTRICAL SAFETY			
Electrical Leakage, etc.	E, D	E, D	E, D
Ground Fault Interrupter	D	D	D
OTHER			
Lightning Intensity	E	E	E
UV Intensity	C, E	C, E	C, E
Noise Level	E	E	E
Vibration	E	E	E

Key for Table 2.

Req.	Required during certification
A	Required for proper certification if the cabinet is new, or has been moved or if panels have been removed for maintenance
B	If used with gloves
C	If present
D	Encouraged for electrical safety
E	Optional, at the discretion of the user
F	Used to determine air distribution within cabinet for clean to dirty procedures
N/A	Not applicable

EH&S will maintain a database of certifications and notify PI's/Users when cabinet requires certification.

Moving Biological Safety Cabinet or Laminar Flow Hoods

No Biological Safety Cabinet or laminar flow hood will be relocated unless previously decontaminated by an outside vendor.

Approved Vendors

EH&S will approve all outside vendors certifying Biological Safety Cabinets.

CHAPTER 16 - BIOLOGICAL WASTE

Biohazardous waste must be handled and disposed of in a manner, which promotes the safety of patients, employees and the community. See Appendix A for waste stream schematic drawing, illustrating ultimate disposal of all wastes.

The purpose of this procedure is to eliminate downstream exposures and reduce the risk and/or spread of infection by the safe handling and disposal of regulated and non-regulated wastes.

Biohazardous Waste

Biohazardous waste is either regulated under N.C. Medical Waste regulations or the OSHA rules. Biohazardous wastes at the WFUSM include:

- liquid or semi-liquid blood or other body fluid in amounts ≥ 20 ml per container;
- blood or any other body fluid in individual containers that CANNOT be emptied;
- contaminated sharps, including syringes, needles and scalpel blades;
- pathological wastes containing blood or any other body fluid, human tissues, organs and body parts, and animal carcasses or body parts if exposed to pathogens in research, in the production of biologicals or in vivo testing, or if they died of a known or suspected infectious disease;
- Microbiological wastes containing blood or other potentially infectious materials, cultures, etiological agents, and laboratory specimens.
- contaminated* items that would release blood or any other body fluid or tissue in a liquid or semi-liquid state if compressed;
- soiled dressings, soiled gloves, tubings, and containers that have held blood or any other body fluid, secretion or excretion;
- Items that are caked with dried blood or any other body fluid or tissue capable of releasing these materials during handling.

The term "Contaminated" means the presence or reasonably anticipated presence of blood or any other body fluid or tissue on an item or surface.

Non-Regulated Wastes

"Non-regulated wastes" at the Medical Center include:

- wastes containing food scraps or other decomposable materials;
- rubbish, non-recyclable paper, cardboard, and non-contaminated disposable articles;
- urine and feces

Packaging and Labeling

Categories, packaging and labeling requirements, and ultimate disposal of Biohazardous and non-regulated waste streams are shown in the schematic drawing ([Appendix A](#)) attached to this policy. These waste streams apply regardless of any isolation status.

All waste containers:

- must be leakproof receptacles that can be thoroughly cleaned and maintained in a sanitary manner,
- must be lined with a plastic bag,
- must be emptied as often as necessary to maintain sanitary conditions.

Handling and Disposal of Biohazardous Wastes

Liquid or semi-liquid blood or other body fluid must be:

- Handled cautiously in order to avoid splashing or creating aerosols when disposing of human excretions and secretions.

NOTE: Cautious handling includes: Use of APPROPRIATE PERSONAL PROTECTIVE EQUIPMENT and pouring liquids in a manner that reduces splashing

- Disposed of down a drain connected to a sanitary sewer (i.e. toilet or hopper). All blood/body fluids that can be flushed down the sewer MUST BE. These wastes must NEVER be poured into or flushed down a sink used for handwashing or other clean functions. The individual disposable containers that have had blood/body fluid emptied from them should be handled as OSHA regulated wastes.

Individual disposable containers that hold blood or body fluids in quantities of 20 ml or more AND CANNOT BE EMPTIED (i.e. atrium chest tube drainage systems), must::

- Have the connecting tubing clamped off.
- Be placed in a small red biohazard bag at the bedside, tied off and removed from the patient's room to the dirty utility room for disposal in the red biohazard labeled waste container. Red biohazard waste containers are checked daily for pick up by Facility Services personnel who tape the liner closed and interlock the box top for transportation. Box is labeled with the type of waste and the handler's initials.

Sharps

Contaminated sharps, syringes, needles, scalpel blades, and any contaminated object that can penetrate the skin including broken glass, broken capillary tubes, and exposed ends of dental wires must be:

- Handled with caution, manipulated as little as possible and disposed of in an approved sharps container as soon as practical. Contaminated needles and other contaminated sharps will not be sheared or broken. They must also not be purposely bent, recapped, removed, or manipulated by hand EXCEPT as noted below:
 - ✓ When there is no feasible alternative (i.e. no sharps box in room, or sharps box overfilled).
 - ✓ When such action is required by a specific medical procedure (i.e. for local anesthesia). When such recapping or needle removal is approved, it must be accomplished through the use of a mechanical device or a one-handed technique if no mechanical means are available.
- Any type of sharps requiring containment (i.e. disposable scissors, broken glassware, syringe on floor or other sharp objects too large for regular sharps container) which may be contaminated must not be picked up directly with hands. Facilities Services personnel will pick up or clean up using mechanical means such as a brush and dust pan, tongs, or forceps. Puncture-resistant containers for broken glass (contaminated or not) are labeled as such, provided by Facilities Services and are kept in the soiled utility room on each floor.
- During use, sharps boxes must be:
 - ✓ Easily accessible to personnel;
 - ✓ Located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g. laundry);
 - ✓ Maintained upright throughout use; and
 - ✓ Replaced before level of sharps passes maximum 'fill line'. There is no recommended interval for changing sharps boxes. Instead boxes should be changed before the level of sharps passes the "Maximum Fill Line" and should also be changed any time they become offensive by odor or sight.
- All contaminated sharps and syringes with or without needles should be placed immediately, or as soon as feasible, after use in containers that are:
 - ✓ Puncture resistant;
 - ✓ Leakproof on sides and bottom;
 - ✓ Labeled with closable;
 - ✓ A biohazard label;
 - ✓ Constructed so that they will not spill their contents if knocked over and will not themselves allow injuries when handled.
 - ✓ Sharps containers must never be placed in the trash or linen chutes.
- In addition to previously listed requirements., REUSABLE sharps contaminated with blood or any other body fluid or tissue or material should be:
 - ✓ Stored or processed in a manner that does not require employees to reach by hand into containers where these sharps have been placed.

- ✓ Placed in appropriate containers that cannot be emptied or opened until properly processed. These containers cannot be cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury.
- When moving containers of contaminated sharps from the area of use, the containers shall be:
 - ✓ Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping;
 - ✓ Placed in a secondary container if leakage is possible. The second container must be:
 - Closable;
 - Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and
 - Labeled with a biohazard label.

Pathological Waste

Pathological wastes containing blood or any other body fluid, human tissues, organs and body parts, and animal carcasses or body parts if exposed to pathogens in research, used in the production of biologicals or in vivo testing, or if they died of a known or suspected infectious disease should be handled by:

- Using extreme caution, utilize Personal Protective Equipment (PPE) when necessary.
- Wrapping of these items in a red biohazard bag (small ones in Par Stock, PPE Cart or General Stores). If the body part is small, use several paper towels or a piece of cardboard as filler in the bag so that the biohazard label can be seen after the bag is closed. Tape the bag tightly closed. Complete the Pathology Request form for body parts disposal.
- Hand delivering properly packaged body parts to the Surgical Pathology freezer behind the Stat Lab. At no time prior to delivery to Surgical Pathology are body parts to be left unattended.
- Body parts are ultimately incinerated.

Microbiological Waste

Microbiological wastes containing coagulated blood or any other body fluid, cultures, etiological agents, and laboratory specimens must be:

- Handled with caution, utilizing PPE when necessary.
- Gathered in a polypropylene biohazard-labeled bucket lined with a single clear or orange autoclave bag that is open.
- Steam autoclaved for 60 min. (for 15 LB/bucket maximum with as many buckets as fit without stacking)
- Tied off into first bag.
- Placed in a second brown plastic bag and tied off.
- In order to transport the bags from the autoclave, they must be placed in a rigid container for ultimate disposal in landfill.

Contaminated Items

Contaminated items that would release blood or any other body fluid in a liquid or semi-liquid state if compressed must be:

- Handled and disposed of with caution. Depending upon the type of handling/procedure, personal protective equipment (PPE) may need to be worn.
- Placed in containers which are:
 - ✓ Closable;
 - ✓ Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping; and
 - ✓ Labeled with a biohazard label
- These containers must be closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport or shipping.
- If outside contamination of the primary regulated waste container occurs, it must be placed in a second container.

Items with less than 20 ml blood

Items with less than 20 ml of blood or drainage (i.e. individual disposable containers that have had blood or body fluids emptied from them), as well as tubes that have been removed from the patient, soiled dressing, and soiled gloves must be:

- Placed in the white biohazard-labeled trash liner at the patient's bedside;
- Drains/tubes and other aesthetically offensive items should be tied off in the bag and taken from the patient's room to the soiled utility room as soon as possible.

Items Caked with Dried Blood

Items that are caked with dried blood or any other body fluid or tissue capable of releasing these materials during handling must be:

- Handled with caution, utilizing PPE when procedures call for them, and placed in a white biohazard-labeled bag if disposable.
- Examined prior to servicing or shipping and decontaminated as necessary (see "Sanitation" PPB-GS-IC-92 98-32 for approved types of disinfectants) unless the manufacturer prohibits same.
- Biohazard-labeled in a readily observable place, stating which portions remain contaminated.

Disposal of Non-regulated Waste

Handling and disposal of non-regulated wastes includes:

- Disposal with sufficient frequency and in such a manner as to prevent a nuisance (odor or pests).
- Wastes containing food scraps or other decomposable materials must be kept covered with tight-fitting lids when filled or stored, or not in continuous use.
- Rubbish, non-recyclable paper, cardboard, and non-contaminated disposable articles must be placed in plastic-lined trash cans throughout the facility, collected by facilities services personnel on a routine basis, and disposed of in the landfill;

Urine and feces must be:

- Handled cautiously in order to avoid splashing or creating aerosols when disposing of human excretions/secretions. NOTE: Cautious handling includes pouring liquids in a manner that reduces splashing and averting face during process of pouring. APPROPRIATE PERSONAL PROTECTIVE EQUIPMENT MUST BE USED (i.e. gown, gloves, and face shield or respirator and goggles).
- Poured or flushed down a drain connected to sanitary sewer (i.e. toilet or hopper). All human waste must be disposed of in this manner and must NEVER be poured into or flushed down a sink used for handwashing or other clean functions. Non-liquid feces in linen or diapers must be emptied into a toilet or hopper.
- The individual containers that have had these wastes emptied from them should be rinsed clean before reuse by the same patient if reusable. If they cannot be rinsed clean, they should be sent for reprocessing and replaced with a clean item. Disposable items that cannot be rinsed clean should be discarded as an OSHA regulated waste and replaced with a clean item.

Labeling

Warning labels for Biohazardous Waste must include the universal biohazard symbol and:

- Be affixed to containers of regulated waste, refrigerators and freezers containing blood or any other body fluid/material.

- Be affixed to other containers used to store, transport or ship blood or any other body fluid/material EXCEPT:
 - ✓ When red bags or red containers are used
 - ✓ When containers of blood, blood components, or blood products that are labeled as to their contents have been released for transfusion or other clinical use
 - ✓ When individual containers of blood or any other body fluid/material are placed in a labeled container during storage, transport, shipment or disposal.
- Be fluorescent orange or orange-red with lettering or symbols in a contrasting color.
- Be affixed onto the container by adhesive or other method that prevents their loss or unintentional removal.
- Be used in order to protect all employees and service or manufacturing representatives who service, handle, prepare for or receive from shipping, equipment that is contaminated with blood or other potentially infectious materials. They must either be:
 - ✓ Cleaned appropriately (see ppb-gs-ic-92-98-32, "sanitation") after use

OR

- ✓ Be examined prior to servicing or shipping and be decontaminated unless that is not feasible. If the item cannot be decontaminated (or parts of it cannot be) it must have a biohazard label affixed to the equipment with a readily observable, legible label that states which portions of the equipment remain contaminated.

Biohazardous Waste and Non-regulated Trash Disposal Guidelines

BUFF OR BROWN BAG WASTE:

Offices not related to patient care (i.e. not generating contaminated or infectious waste) will routinely be stocked with these liners.

Use the **BUFF OR BROWN BAGS** (with no Biohazard labels) for the disposal of non-patient care paper, cups, etc.

No Glass or Radioactive Waste can be placed in these bags/containers.

RED BAG WASTE:

RED BAGS are placed in the BFI boxes by Housekeeping, in locations determined by each area. Clinic and laboratory staff is responsible for:

- Placing a bar code label on each box, and fill in the date when closed;
- Closing the **RED BAGS** as follows: a) twist the top, b) fold over the twisted "handle", and c) tape the fold securely.

WFUSM Environmental Services will seal the box, remove it and replace it with a new box and bag.

Use the **RED BAGS** (which have the biohazard labels) for the disposal:

Items saturated with or containers which contain 20 cc (a little less than $\frac{3}{4}$ ounce) or more of blood or body fluids. Liquids (blood and body fluids) that can be flushed down the dirty utility area hopper, should be flushed (wearing the appropriate protective wear), whenever possible.

Pathological Waste (human tissues, organs and body parts; carcasses and body parts of animals exposed to pathogens potentially dangerous to humans, were used in production of biologicals or in vivo testing or pharmaceuticals, or died of a known or suspected disease transmissible to humans).

Microbiological Waste (cultures, stocks, specimens of infectious agents). **Note:** Autoclaving and disposal in the regular trash is an acceptable alternative for these waste materials (except for blood products) and these are placed in an **ORANGE** autoclave bag.

To package full sharps and chemotherapy containers, the bar codes on all boxes that contain Chemotherapy must have a yellow stripe/mark through the bar code on the exterior.

No Glass or Radioactive Waste can be placed in these bags/containers.

WHITE BAG WASTE:

Areas where blood and body fluids are common will be stocked routinely with the **WHITE BAGS** (which have the Biohazard labels) to facilitate compliance. Housekeeping will replace the **WHITE BAGS** daily. If more frequent changes are needed, staff will be responsible for these.

Use the **WHITE BAGS** (with the Biohazard label) for the disposal of patient care items in the clinic and items, which contain less than 20 cc of blood and body fluids.

No Glass or Radioactive Waste can be placed in these bags/containers.

CLEAR BAG WASTE:

Long-lived solids with H-3, C-14, Co-57, or any isotope with a half-life or greater than 90 days must be sealed in a 4-6 mil plastic bag. Long-lived solid waste must contain no liquids including scintillation fluids, carcasses, tissue, blood or blood products. Source vials of long-lived isotopes if not empty and dry should be segregated from the rest of the solid waste and presented separately at disposal time.

Short-lived solids with P-32, P-33, I-125, I-131, Cr-51, S-35, or any isotope with a half-life of less than 90 days must be sealed in 4-6 mil plastic bags. All radioactive materials or radiation warning tape must be removed prior to disposal. I-125, I-131, P-33, and Cr-51 solid waste may be combined. P-32 and S-35 solid waste should be packaged separately. Source vials, whether dry or containing small volumes of liquid, may be placed in bags with short-lived solid waste provided all radioactive materials or radiation-warning labels are removed or defaced. All bags must be securely sealed.

Scintillation vials containing radioactive scintillation media should be collected in 4-6 mil plastic bags in volumes not to exceed 0.5 cubic feet per bag. Scintillation vials containing S-35 or P-32 should be segregated and presented separately. All radioactive materials or radiation warning tape must be removed prior to disposal. All bags must be securely sealed.

Carcasses should be double-bagged in 4-6 mil plastic bags and, when possible, frozen prior to disposal through Radiation Safety. Tissue, blood and blood products, and associated bedding are considered as "carcass". All bags must be securely sealed. All radioactive materials or radiation warning tape must be removed prior to disposal.

Sharps

All contaminated sharps (glass slides, needles, etc) must be placed in a puncture resistant container, such as on the SAGE disposable units or a glass disposal box, prior to placing these in a **RED BAG**. For information, contact WFUSM Housekeeping (716-4417) or WFUSM Environmental Health and Safety (716-9375).

All clean glass must be placed in a puncture resistant container manufactured for glass disposal.

CHAPTER 17 - AUTOCLAVES

The use of an autoclave is a very effective way to decontaminate infectious waste. Autoclaves work by killing microbes with superheated steam. Recently, there have been several accidents involving the use of autoclaves on campus. In an effort to raise user awareness in the University community, the EH&S offers the following safety tips:

- Do not put sharp or pointed contaminated objects into an autoclave bag. Place them in an appropriate rigid sharp disposal container.
- Use caution when handling an infectious waste autoclave bag, in case sharp objects were inadvertently placed in the bag. Never lift a bag from the bottom to load it into the chamber. Handle the bag from the top.
- Do not overfill an autoclave bag. Steam and heat cannot penetrate as easily to the interior of a densely packed autoclave bag. Frequently the outer contents of the bag will be treated but the innermost part will be unaffected.
- Do not overload an autoclave. An overpacked autoclave chamber does not allow efficient steam distribution. Considerably longer sterilization times may be required to achieve decontamination if an autoclave is tightly packed.
- Conduct autoclave sterility testing on a regular basis using appropriate biological indicators (*B. stearothermophilus* spore strips) to monitor efficacy. Use indicator tape with each load to verify it has been autoclaved.
- Do not mix contaminated and clean items together during the same autoclave cycle. Clean items generally require shorter decontamination times (15-20 minutes) while a bag of infectious waste (24" x 36") typically requires 45 minutes to an hour to be effectively decontaminated throughout.
- Always wear personal protective equipment, including heat-resistant gloves, safety glasses and a lab coat when operating an autoclave. Use caution when opening the autoclave door. Allow superheated steam to exit before attempting to remove autoclave contents.
- Be on the alert when handling pressurized containers. Superheated liquids may spurt from closed containers. Never seal a liquid container with a cork or stopper. This could cause an explosion inside the autoclave.
- Agar plates will melt and the agar will become liquefied when autoclaved. Avoid contact with molten agar. Use a secondary tray to catch any potential leakage from an autoclave bag rather than allowing it to leak onto the floor of the autoclave chamber.
- If there is a spill inside the autoclave chamber, allow the unit to cool before attempting to clean up the spill. If glass breaks in the autoclave, use tongs, forceps or other mechanical means to recover fragments. Do not use bare or gloved hands to pick up broken glassware.
- Do not to leave an autoclave operating unattended for a long period of time. Always be sure someone is in the vicinity while an autoclave is cycling in case there is a problem.

Autoclaves must be placed under preventive maintenance contracts to ensure they are operating properly. If you have any questions about maintenance and use of autoclaves, please contact EHS at 716-6440.

CHAPTER 18 - BIOSAFETY AUDITS

Biosafety Audits are incorporated into the laboratory audit and benchmarking (LAB) process. Areas where biohazardous research is being conducted are included in these audits. The primary purpose is to ensure that the health, safety and security aspects of the research protocol are being followed.

It is the policy of Wake Forest University Health Sciences that laboratories where biohazardous materials are being used must be inspected:

- Whenever a Biosafety Application is submitted to the committee.
- Annually thereafter.
- Select agent users involving biologicals, quarterly inspection.

This chapter provides a uniform Laboratory Biosafety Audit to be used by EH&S when:

- Conducting assessments of laboratories in response to submission of Biosafety Applications.
- Conducting annual assessment of laboratories with valid Biosafety Applications.
 - ✓ The frequency of inspection for laboratories that use Recombinant DNA techniques shall be annually.
 - ✓ The frequency of inspection for laboratories that use other Biohazardous Agents shall be annually.

Biosafety Inspections shall cover the following areas:

- Any and all conditions of the Biosafety Application
- Laboratory safety
- Biosafety
- Security

Inspection Follow-up

Initial Inspection (pursuant to Biosafety Application)

- The Office of Environmental Health and Safety shall provide a copy of the laboratory inspection report and recommendations to approve/not approve to the Chairman of the Biosafety Committee.
- A copy of the inspection report shall be forwarded by the Director of Environmental Health and Safety to the principal investigator

Annual Inspections

- The Office of Environmental Health and Safety shall forward a copy of the inspection report to the principal investigator.

- The principal investigator shall submit a corrective action summary within 30 days.
- Reinspections will be scheduled within 30 days to verify corrective action has been instituted by the Principal Investigator.
- If a repeat violation of the same item is observed within a calendar year, the Authorized User's department chair will receive a copy of the inspection report.
- If a third violation of the same item occurs within a calendar year, the Authorization will be suspended until the User meets with the Chair of the Biosafety Committee to justify continued use of Biohazardous materials. The Committee and the Associate Dean will be notified of the results of this meeting.

Committee action may include (but is not limited to):

- Re-training of laboratory employees,
- Placement of the User into a probationary status that will include increased frequency of EH&S Biosafety audits,
- Limitation or suspension of the Authorization, or
- Termination of the Authorization and removal of all biohazardous materials EH&S.

Annual Review and Report

The Director of Environmental Health and Safety shall report annually to the Biosafety Committee concerning the effectiveness of the Inspection Program. The report shall include:

- Number of authorized users
- Number of inspections (Initial and Annual)
- Number of situations requiring corrective action.
- Percentage of corrective actions completed.
- Program areas requiring improvement (documentation, inspections, resources, etc.) and recommendations.

The committee shall recommend to the Director of EH&S any areas which it feels need improvement.

CHAPTER 19 - BIOLOGICAL EMERGENCY PLAN

Biological Emergency Plan

Wake Forest University School of Medicine

EMERGENCIES BETWEEN 8 A.M. – 5 P. M. MONDAY - FRIDAY

Contact EH&S Industrial Hygiene/Chemical Safety at 716-1222.

EMERGENCIES AFTER 5 P. M. MONDAY – FRIDAY, WEEKENDS AND HOLIDAYS

Call Safety/Security at 716-3305.

Report that you have a biohazard emergency.

Give the operator your name, location and telephone number.

Safety/Security will contact the appropriate individuals.

MINOR SPILLS

Spills of Class 1 organisms

Spills of high-risk organisms (Class 2 and Class 3) inside a BSC.

Spills of high risk organisms (Class 2 and Class 3) < 10 ml outside a BSC.

Spills of blood and body fluids

MAJOR SPILL

Spills of high-risk organisms (Class 2 and Class 3) > 10 ml outside a BSC.

STRUCTURE/ROSTER OF EMERGENCY TEAM

Industrial Hygiene Officer

Industrial Hygienist II

Industrial Hygienist II

REQUIRED EQUIPMENT

Disinfectant

Mops

Tyveks

Blood spill kit

Gloves

Goggles

DISPATCH OF EMERGENCY TEAM

- When accidents occur that involves the mishandling or escape of biohazardous materials, the principal investigator or laboratory supervisor is to be notified immediately.
- Spills of high risk organisms (Class 2 and all Class 3) shall be reported to the Industrial Hygiene Officer at 716-1222 during normal working hours
- Safety/Security at the emergency telephone number 716-3305 after normal working hours by the principal investigator or laboratory supervisor. The Safety/Security Section will contact the Industrial Hygiene Officer for appropriate response. All employees and/or students have an obligation to themselves and their colleagues to report accidents immediately in order to minimize potential hazard.
- When a biohazardous spill also involves radioactivity, cleanup procedures may have to be modified. The extent of the modification will depend on the level of radiation and the nature of the isotope involved. The Radiation Safety Officer should be called during normal working hours at 716-1202 or the Safety/Security after 5:00 PM or on weekends at 716-3305.

ISOLATION/ASSESSMENT OF HAZARD

Safety/Security

Determine type of material spilled and quantity

Type of Material	Quantity	Required Response
Blood or other potentially contaminated materials	Any	Minor – Contact WFUSM Housekeeping
Biohazard materials		
Inside Biological Safety cabinet		Minor
Class 1 Organism – Outside Biological Safety Cabinet		Minor
Class 2 or 3 Organisms – Outside Biological Safety Cabinet	< 10 ml	Minor
Class 2 or 3 Organisms – Outside Biological Safety Cabinet	>10 ml	Major – Contact WFUSM EH&S Dispatch Security to secure perimeter

AMELIORATION/REMEDIATION ACTIVITIES

Minor Spills

- Blood and other Body Fluids
- Biohazard Spills Inside Laminar Flow Biological Safety Cabinets (LFBSC)
 - ✓ The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory as long as the spilled materials are contained in the biological safety cabinet. Decontamination of the work zone can usually be effected by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Gaseous decontamination may be required to clean up the interior sections of the cabinet.
 - ✓ Chemical decontamination procedures should be initiated immediately while the biological safety cabinet continues to operate. Continuing the operation of the LFBSC helps to prevent the escape of contaminants from the cabinet.
 - ✓ Wearing protective gloves spray or wipe walls, work surfaces, and equipment with an appropriate decontaminating solution. A disinfectant detergent, such as Wescodyne or Environ has the advantage of detergent action on extraneous organic substances, which may interfere with the microbicidal activity of the disinfectant.
 - ✓ Flood tray top, drains pans, and catch basins below work surface with decontaminating solution and allow to stand for 20 minutes.
 - ✓ Drain excess decontaminating solution from tray and drain pans into cabinet base. Lift out tray and removable exhaust grille work. Clean the top and bottom (underside) surfaces using a sponge or clean cloth soaked in decontaminant solution. Following the cleaning process, replace the tray and grille work in their proper position. Place gloves and sponge or cloth in autoclave pan and autoclave these items.
 - ✓ Drain decontaminating solution from cabinet base into appropriate container and autoclave according to standard procedures.
 - ✓ If gaseous decontamination of the cabinet's interior sections is needed, call the Industrial Hygiene Officer at 716-1222.
- Biohazard Spills Outside Laminar Flow Biological Safety Cabinets (LFBSC) Class 1 organisms and Class 2 and 3 Organisms < 10 ml

Spills (less than 10 ml and generating little aerosol) on equipment, laboratory benches, walls, or floors:

- ✓ Warn all personnel not essential for spill containment to stay clear of the contaminated area. This may be accomplished verbally or, when appropriate, by posting warning signs on the doors.
- ✓ Thoroughly wash hands and other apparently contaminated areas with soap and water. Put on clean disposable gloves. Surgical gloves or Trutouch disposable gloves may be ordered through Central Research Stores.
- ✓ Cover the spill area with paper towels soaked in appropriate decontamination solution. (See Attachment A)

- ✓ Wipe up the spill with the soaked paper towels and place the used towels in an autoclave pan and autoclave.
- ✓ Pour decontaminating solution around and on the area of the spill. Let stand for 20 minutes then wipe up with paper towels. Place gloves and paper towels in autoclave pan and autoclave.
- ✓ Wash hands and other apparently contaminated areas again with soap and water.
- Major Spills Class 2 or 3 organisms (more than 10 ml or with considerable aerosol):
 - ✓ Close laboratory doors and post warning signs to prevent others from entering the laboratory.
 - ✓ Wash hands and other apparently contaminated areas with soap and water.
 - ✓ Report the accident to the Supervisor and to the Industrial Hygiene Officer, 716-1222.
 - ✓ If personal clothing is contaminated, remove all outer clothing and place it in autoclave or container for autoclaving. Put on clean garments.
 - ✓ Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.
 - ✓ Upon returning to the laboratory to start decontamination, check to see if laboratory doors are closed and appropriate signs are displayed. Put on surgical gloves. Respirators or other safety equipment may be required, depending on the microorganism involved. Check with the Principal Investigator or Laboratory Supervisor or Industrial Hygiene Officer.
 - ✓ Pour a decontamination solution (See Attachment A) around the spill and allow this solution to flow into the spill. Paper towels soaked with decontamination solution may be used to cover the area. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.
 - ✓ Let decontamination solution – microorganism mixture stand for 20 minutes or longer to allow adequate contact time.
 - ✓ Using autoclave dust pan and squeegee transfer all contaminated materials to deep autoclave pan, cover with suitable cover, and autoclave according to standard directions.
 - ✓ Place dustpan squeegee in an autoclavable bag and autoclave according to standard directions.
 - ✓ Remove gloves and other contaminated garments and place them in an autoclave container for autoclaving.
 - ✓ Thoroughly wash hands, face, and other apparently contaminated areas.

CLEANUP/MOPUP ACTIVITIES

Special care in decontamination may necessary. The Principal Investigator and/or the Industrial Hygiene Officer may require the collection of sample cultures to determine that the area has been effectively decontaminated.

Report spill to Biosafety Committee.

EQUIPMENT ACTIVITIES

Replace and clean supplies/equipment used in response.

TRAINING

Group	Training Required			
	Blood	Inside BSC	< 10 ml	> 10 ml
EH&S Industrial Hygiene/Chemical Safety	X	X	X	X
Principal Investigator	X	X	X	-
Laboratory Technician	X	X	X	-
WFUBMC Hazardous Materials Team	X	X	X	X

ANNUAL PROGRAM REVIEW

This document shall be reviewed annually by the Industrial Hygiene Officer and recommendation made to the Biosafety Committee concerning program improvements and deficiencies. An annual report of Biological Emergency Responses shall be made to the committee.

APPENDIX A - [TABLE OF RECOMBINANT DNA APPROVAL LEVELS](#)

This appendix provides a reference table for IBC and NIH approvals for recombinant DNA research.

APPENDIX B - LIST OF BIOSAFETY LEVEL 2, 3 AND 4 AGENTS

This appendix provides a listing of Biosafety Level 2 and 3 agents. It is a reference to supplement existing CDC documents and the biosafety program. A more inclusive listing may be found at <http://www.absa.org/riskgroups/>

Bacterial Agents BL2
Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)
Actinobacillus
Actinomyces pyogenes (formerly Corynebacterium pyogenes)
Aeromonas hydrophila
Amycolata autotrophica
Archanobacterium haemolyticum (formerly Corynebacterium haemolyticum)
Arizona hinshawii-all serotypes
Bacillus anthracis
Bartonella henselae, B. quintana, B. vinsonii
Bordetella including B. pertussis
Borrelia recurrentis, B. Burgdorferi
Burkholderia (formerly Pseudomonas species)
Campylobacter coli, C. fetus, C. jejuni
Chlamydia psittaci, C. trachomatis, C. pneumoniae
Clostridium botulinum, Cl. Chauvoei, Cl. haemolyticum, Cl. Histolyticum, Cl. novyi, Cl.
Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix rhusiopathiae
Escherichia coli-all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli-0157:H7
Haemophilus ducreyi, H. influenzae
Helicobacter pylori
Klebsiella-all species except K. oxytoca
Legioella including L. pneumophila
Leptospira interrogans-all serotypes
Listeria
Moraxella
Mycobacterium including M. avium complex, M. asiaticum, M. bovis BCG vaccinestrain, M. chelonae, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi
Mycoplasma, except M. mycoides and M. agalactiae
Neisseria gonorrhoea, N. meningitidis
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
Rhodococcus equi
Salmonella including S. arizonae, S. choleraesuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi, A,B,C,S. typhi, S. typhimurium
Shigella including S. boydii, S. dysenteriae, type 1, S. flexneri, S. sonnei
Sphaerophorus necrophorus

Bacterial Agents BL2
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus including <i>S. pneumoniae</i> , <i>S. pyogenes</i>
Treponima pallidum, <i>T. carateum</i>
Vibrio cholerae, <i>V. parahemolyticus</i> , <i>V. vulnificus</i>
Yersinia enterocolitica

Fungal Agents BL2
Blastomyces dermatitidis
Cladosporium bantianum, <i>C. (Xylohypha) trichoides</i>
Cryptococcus neoformans
Dactylaria galopava (<i>ochroconis gallopavum</i>)
Epidermophyton
Exophiala (<i>Wangiella</i>) dermatitidis
Fonsecaea pedrosoi
Microsporum
Paracoccidioides braziliensis
Penisillium marneffeii
Sporothrix schenckii
Trichophyton

Parasitic Agents	BL2
Ancylostoma human hookworms including <i>A. duodenale</i> , <i>A. ceylanicum</i>	
Ascaris including <i>Ascaris lumbricoides</i> suum	
Babesia including <i>B. divergens</i> , <i>B. microti</i>	
Brugia filaria worms including <i>B. malayi</i> , <i>B. timori</i>	
Coccidia	
Cryptosporidium including <i>C. parvum</i>	
Cysticercus cellulosae (hydatid cyst, larva of <i>T. solium</i>)	
Echinococcus including <i>E. granulosus</i> , <i>E. multilocularis</i> , <i>E. vogeli</i>	
Enterobius	
Entamoebaa histolytica	
Fasciola including <i>F. gigantica</i> , <i>F. hepatica</i>	
Giardia including <i>G. lamblia</i>	
Heterophyes	
Hymenolepis including <i>H. diminuta</i> , <i>H. nana</i>	
Isospora	
Leishmania including <i>L. Braziliensis</i> , <i>L. donovani</i> , <i>L. ethiopia</i> , <i>L. major</i> , <i>L. mexicana</i> , <i>L. peruviana</i> , <i>L. tropica</i>	
Loa loa filaria worms	
Microsporidium	
Naegleria fowleri	
Necator human hookworms including <i>N. americanus</i>	
Onchoerca filaria worms including, <i>O. volvulus</i>	
Plasmodium including simian species, <i>P. cynomologi</i> , <i>P. falciparum</i> , <i>P. malariae</i> , <i>P. ovale</i> , <i>P. vivax</i>	
Sarcocystis including <i>S. sui hominis</i>	
Schistosoma including <i>S. haematobium</i> , <i>S. intercalatum</i> , <i>S. japonicum</i> , <i>S. mansoni</i> , <i>S. mekongi</i>	
Strongyloides including <i>S. stercoralis</i>	
Taenia solium	
Toxocara including <i>T. canis</i>	
Toxoplasma including <i>T. gondii</i>	
Trichinella spiralis	
Trypanosoma including <i>T. brucei brucei</i> , <i>T. brucei gambiense</i> , <i>T. brucei rhodesiense</i> , <i>T. cruzi</i>	
Wuchereria bancrofti filaria worms	

Viruses	BL2
Adenoviruses, human-all types	
All human papilloma viruses	
Alphaviruses (Togaviruses)-Group A Arboviruses	
Arena viruses	
Bunyamwera virus	
Calciiviruses	
Coronaviruses	
Coxsackie viruses types A and B	
Cytomegalovirus	
Dengue virus serotypes 1,2,3, and 4	
Eastern equine encephalomyelitis virus	
Echoviruses - all types	
Epstein Barr virus	
Flaviviruses (Togaviruses) - Group b Arboviruses	
Hepatitis A, B, C, D, and E viruses	
Herpes simplex types 1 and 2	
Herpes zoster	
Herpesviruses-except herpesvirus simiae (Monkey B virus)	
Human herpesvirus types 6 and 7	
Human parvovirus (B19)	
Influenza viruses types A, B, and C	
Lymphocytic choriomeningitis virus (non-neurotropic strains)	
Measles virus	
Mumps virus	
Newcastle disease virus	
Orthomyxoviruses	
Other tick-bourne orthomyxoviruses	
Papovaviruses	
Parainfluenza viruses types 1,2,3, and 4	
Paramyxovviruses	
Parvoviruses	
Picornaviruses	
Polioviruses - all types, wild and attenuated	
Poxviruses - all types except Monkeypox virus	
Rabies virus - all strains	
Reoviruses-all type indcluding Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)	
Respiratory syncytial virus	
Rhabdoviruses	
Rhinoviruses - all types	
Rift Valley fever virus vaccine strain MP-12	
Rubivirus (rubella)	
Tacaribe virus complex	
Togaviruses	
Venezuelan equine encephalomyelitis vaccine strain TC-83	
Vesicular stomatitis virus - laboratory adapted strains including VSV-	

Viruses BL2
Indiana, San Juan, and Glasgow
Western equine encephalomyelitis virus
Yellow fever virus vaccine strain 17D

Bacterial Agents Including Rickettsia BL3
Bartonella
Brucella including B. abortus, B. canis, B. suis
Burkholderia (Pseudomonas) mallei, B. pseudomallei
Coxiella burnetii
Francisella tularensis
Mycobacterium bovis (except BCG strain)
Pasteurella multocida type B - "buffalo" and other virulent strains
Rickettsia akari, R. australis, R. Canada, R. conorii, R. prowazekii, R. rickettsii, R. siberica, R. tsutsugamushi, R. typhi (R. mooseri)
Yersinia pestis

Fungal Agents BL3
Coccidioides immitis (sporulating cultures; contaminated soil)
Histoplasma capsulatum, H. capsulatum var. dubosii

Viruses and Prions BL3
Alphaviruses (togaviruses) - Group A arboviruses
Arenaviruses
Bunyaviruses
Flaviviruses (togaviruses) - Group B Arboviruses
Hantaviruses including Hantaan virus
Human immunodeficiency virus (HIV) types 1 and 2
Human T cell lymphotropic virus (HTLV) types 1 and 2
Japanese encephalitis virus
Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
Monkeypox virus
Poxviruses
Prions
Retroviruses
Rhabdoviruses
Rift Valley fever virus
Semliki Forest virus
Simian immunodeficiency virus (SIV)
St. Louis encephalitis virus
Transmissible spongiform encephalopathies (TME) agent (Creutzfeldt-

Viruses and Prions BL3
Jacob disease and kuru agents)
Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83)
Vesicular stomatitis virus
Yellow fever virus

Viral Agents BL4
Central European tick-borne encephalitis
Congo-Crimean hemorrhagic fever
Ebola*
Guanarito
Junin*
Kyasanur Forest disease
Lassa*
Machupo*
Marburg*
Omsk hemorrhagic fever
Russian Spring-Summer encephalitis
Sabia*

* Export permit required by Department of Commerce.

Animal Viral Etiologic Agents
A containment level appropriate for BL1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for BL2 human agents is recommended.
Avian leukosis virus
Avian sarcoma virus
Baculoviruses
Bovine leukemia virus
Bovine papilloma virus
Feline leukemia virus
Feline sarcoma virus
Gibbon leukemia virus
Herpesvirus saimiri
Herpesviruses
Herpesviruses ateles
Marek's disease virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine cytomegalovirus
Murine leukemia virus
Murine sarcoma virus

Animal Viral Etiologic Agents
Papovaviruses
Polyoma virus
Rat leukemia virus
Retroviruses
Shope papilloma virus
Simian virus 40

APPENDIX C - BIOSAFETY COMMITTEE PROCEDURES

THIS DOCUMENT MAY BE ACCESSED VIA THE LINK ABOVE.

APPENDIX D1 - [BIOSAFETY COMMITTEE APPLICATION](#)

THIS DOCUMENT MAY BE ACCESSED VIA THE LINK ABOVE.

APPENDIX D2 - BIOSAFETY REGISTRATION

THIS DOCUMENT MAY BE ACCESSED VIA THE LINK ABOVE.

APPENDIX E - HUMAN GENE THERAPY

THIS DOCUMENT MAY BE ACCESSED VIA THE LINK ABOVE.

APPENDIX F - LIQUID DISINFECTANTS

Laboratory personnel should be familiar with the various disinfectants that will effectively kills the biohazardous agents being used. The following information is provided to assist in your selection of appropriate disinfectants.

- Alcohols – Ethyl and Isopropyl are good disinfectants for the vegetative forms of bacteria and lipoviruses.
 - ✓ Ethyl Alcohol
 - Use Dilution: 70-95%
 - Inactivates: vegetative bacteria and Lipoviruses, has variable results with non-lipoviruses and is ineffective with bacterial spores.
 - Other Characteristics: flammable, eye irritant, and toxic (TLV – 1000 ppm)
 - ✓ Isopropyl Alcohol
 - Same as for Ethyl Alcohol except the TLV = 400 ppm.
- Chlorine Compounds – The germicidal effect of chlorine compounds is dependent upon the release of hypochlorous acid and is therefore dependent upon the available chlorine. Allow a contact time of from 10 to 30 minutes.
 - ✓ Use Dilution: 500 ppm available chlorine is recommended for vegetative bacteria and most viruses. Chlorine solutions that are neutral or slightly acidic and with a concentration of approximately 2500 ppm are needed for effectiveness against bacterial spores. Undiluted common household bleach (Chlorox) is alkaline with a pH of 8. or greater. Household bleach typically contains 5.25% sodium hypochlorite for 52500 ppm available chlorine.
 - ✓ Other Characteristics: Chlorine compounds are corrosive to metals; leave a residue; irritate the skin, eyes, and respiratory tract, and are toxic. Chlorine compounds are also rapidly inactivated by organic matter. While chlorine compounds are not generally recommended for routine use, undiluted household bleach is frequently used with biological spills.
- Iodophors – The germicidal effect of iodophors is dependent on the free iodine released from the compound in which it is contained. Allow a contact time of 10 to 30 minutes.
 - ✓ Use Dilution: 25 to 1600 ppm of available iodine. Solutions containing 75 to 150 ppm are generally recommended.
 - ✓ Inactivates: vegetative bacteria, fungi and viruses. There is poor activity against bacterial spores.
 - ✓ Other Characteristics: Although iodophors are less harmful to man than chlorine compounds they can irritate the skin and eyes. Iodophors are corrosive (less than chlorine), they leave a residue and may stain. Iodophor stains, however, can be readily removed with solutions of sodium thiosulfate (NA₂ S₂ O₃). As with the chlorine compounds, iodophors are rapidly inactivated by organic matter. One

advantage is that iodophors have a built-in indicator. As long as the solution is brown or yellow it is still active.

- Phenolic Compounds – These are effective against vegetative bacteria (including mycobacterium tuberculosis), fungi, and lipoviruses. Effectiveness against nonlipid viruses is variable depending on the virus. The phenols are ineffective against bacterial spores.
 - ✓ Use Dilutions: 1.0 – 5.0% Solutions containing 0.5 – 2.0% phenol are effective against lipoviruses.
 - ✓ Other Characteristics: Phenols are corrosive and may leave a sticky, gummy residue. Phenolic compounds are irritating to the skin and eyes and are relatively toxic – Phenol TLV for skin is 5 ppm.
- Quaternary Ammonium Compounds – The efficacy of Quaternary Ammonium compounds still generates considerable controversy. Quats are effective in destroying ordinary vegetative bacteria and lipid containing virus but are not effective against pseudomonas, proteus and other gram-negative bacilli. Also, Quats are not effective against bacterial spores at the usual use concentrations of 1:750.
 - ✓ Use Dilutions: 0.1 to 2.0%
 - ✓ Other Characteristics: Quats are surface-active compounds which possess the useful property of lowering the surface tension of the solution. Other advantages include being nontoxic, odorless, nonstaining, noncorrosive to metals and stable. If used at recommended concentrations, Quats are nonirritating.
 - ✓ Quaternary Ammonium compounds are rapidly inactivated by organic matter.
- Formaldehyde Solutions – Formaldehyde in a 5-8% concentration is an effective liquid decontaminant which inactivates vegetative bacteria, bacterial spores, lipid and nonlipid viruses and fungi.
 - ✓ Use Dilutions: 5.0-8.0%
 - ✓ Other Characteristics: The odor and irritating (skin and eyes) and toxic features (TLV = 1.0 ppm) of formaldehyde solutions reduce the desirability of this solution for general use. Formaldehyde solutions are active in the presence of organic matter and do not corrode metal.

APPENDIX G - BIOHAZARD SPILL PROCEDURES FOR INSIDE LAMINAR FLOW BIOLOGICAL SAFETY CABINETS (LFBSC)

- Keep The LFBSC On
- Put On Protective Gloves
- Spray/Wipe Walls, Work Surfaces, And Equipment With Decontaminating Solution
- Floor Tray Top, Drain Pans, And Catch Basins With Decontaminating Solution
- Allow To Stand For 20 Minutes
- Drain Excess Solution Into Cabinet Base
- Lift Out Tray And Removable Exhaust Grille Work
- Clean Top And Bottom Surfaces With Sponge/Cloth Soaked In Decontaminating Solution
- Replace Tray And Grille Work
- Place Gloves, Sponge, Cloth, Etc. In Autoclave Pan
- Drain Decontaminating Solution from Cabinet Base into Autoclavable Containers Autoclave.
- Gaseous Decontamination Is Needed, Contact The EH&S Industrial Hygiene/Chemical Safety At 777-3099.

PLEASE POST THIS CHECKLIST NEAR THE BIOSAFETY CABINET.

APPENDIX H - BIOHAZARD SPILL PROCEDURES FOR OUTSIDE LAMINAR FLOW BIOLOGICAL SAFETY CABINETS (LFBSC) MINOR SPILLS – CLASS 2 ORGANISMS

- Wash Hands and Other Apparently Contaminated Body Parts With Soap and Water.
- Post Warning To Keep Non-Essential Personnel From Spill Area.
- Put On Protective Gloves.
- Cover Spill Area with Paper Towels Soaked in Decontaminating Solution.
- Wipe Up Spill With Soaked Paper Towels
- Place Used Towels In Autoclave Pan.
- Pour Decontaminating Solution around And On Spill Area.
- Let Solution Stand for 20 Minutes
- Wipe Up With Paper Towels
- Place Paper Towels and Gloves in Autoclave Pan
- Wash Hands with Soap and Water
- Autoclave

PLEASE POST THIS CHECKLIST IN THE LABORATORY.

APPENDIX I - BIOHAZARD SPILL PROCEDURES FOR OUTSIDE LAMINAR FLOW BIOLOGICAL SAFETY MAJOR SPILLS – CLASS 2 AND 3 ORGANISMS

- Wash hands and Other Apparently Contaminated Body Parts With Soap and Water.
- Post Warning Signs and Close Laboratory Door.
- Report Spill to Supervisor and Senior Industrial Hygienist.
- If Clothing Is Contaminated, Remove All Other Garments.
- Place Contaminated Clothing in Autoclave Container.
- Put On Clean Garments.
- Leave Laboratory for 20 Minutes.
- Check To See That Laboratory Doors Are Closed And Warning Signs Displayed Upon Returning To Lab.
- Put On Needed Safety Equipment (Disposable Gloves, Respirators, Etc.)
- Place Paper Towels Soaked In Decontamination Solution over the Spill.
- Pour Decontamination Solution around Spill – Allow Solution to Flow into Spill. Do Not Pour Decontamination Solution Into Spill
- Let Stand For At Least 20 Minutes
- Transfer Contaminated Materials to Autoclave Container Using Autoclavable Dust Pan and Squeegee.
- Place dustpan and Squeegee In Autoclave Container.
- Remove Gloves and Other Contaminated Garments and Place in Autoclave Container.
- Wash Face, Hands, And Other Apparently Contaminated Body Parts.
- Autoclave All Materials That Require Autoclaving.

PLEASE POST THIS CHECKLIST NEAR THE BIOSAFETY CABINET.

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