

Yale University

Office of Environmental Health & Safety

Biological Safety Manual



Office of Environmental Health & Safety
135 College Street, 1st Floor, New Haven, CT 06510
Telephone: 203-785-3550 / Fax: 203-785-7588
www.yale.edu/oehs

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Exposure Incident

Report exposure immediately; you may need immediate therapy.

◆ **Needlesticks/puncture wounds:**

Wash the affected area with antiseptic soap and warm water for 15 minutes

◆ **Mucous membrane exposure:**

Flush the affected area for 15 minutes using an eyewash.

For all exposure incidents:

- ◆ Notify Principal Investigator, manager or supervisor (if available) to initiate accident or exposure incident report.
- ◆ Seek medical assistance immediately (within 1-2 hours) from University Health Services, Urgent Visit (432-0123). Medical Area employees may also go to the Yale-New Haven Hospital (Y-NHH) personnel Health Services (688-2462), Room 130, Grace Building from 7:30 a.m. to 4:30 p.m. or the Y-NHH Emergency Room (688-2222) from 4:30 p.m. to 7:30 a.m.

All employees should receive follow up care through Yale Employee Health (432-7978)

Emergency Phone Numbers

Police and Fire, on campus: 111

Yale University Health Services: 432-0123

OEHS Emergency Numbers

8:30 a.m. to 5:00 p.m. Monday-Friday: 785-3555

Other hours: 111

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Forward

This manual has been prepared as an update to the 1976 *Minimum Safety Guidelines for Biological Research at Yale*, and the 1979 *Yale University Biological Safety Manual*. As with the previous manuals, we have provided a core set of biosafety practices and procedures for the safe handling of known biohazards and potentially infectious materials. Relevant sections from the previous manuals have been maintained and updated where necessary.

The manual focuses on Biosafety Levels 1 and 2, as over 99% of Yale laboratories fall within these designations. A separate manual is available for researchers working in Biosafety Level 3 research laboratories. No work with Biosafety Level 4 agents may be conducted at Yale University.

The Yale Office of Environmental Health and Safety, Occupational Health and Safety Section (OHS) Biosafety Program and the requirements for Yale researchers are outlined in the manual. Registration and training information are provided along with details on work practices, safety equipment and facility design. It is the responsibility of the Principal Investigator or Supervisor to ensure that his/her laboratory is in compliance. That responsibility includes identification of the risk or hazards associated with their research and the application of the appropriate safety procedures. Please read the section on responsibilities for additional information.

In the past, the University has also distributed copies of the Centers for Disease Control/National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories* to all Yale research laboratories. The text has served as a functional biosafety manual for the University. This document and other pertinent biosafety training information and training materials are now available on the Office of Environmental Health and Safety Web site (<http://www.yale.edu/oehs/>). New editions or updates will be posted on our Web site when feasible.

We urge you to use the manual as a road map to compliance within your laboratory. Consult the sections relevant to your research and apply the appropriate safety procedures. The Biosafety Office is available for consultation if you have any question or concern with any aspect of the Biosafety Program at the University. The Occupational Health and Safety training credo, "Think before you act," and "If you do not know, ask," are relevant to the use of this manual. If you are unsure of a requirement or biosafety practice, please contact the Biosafety group at 785-3550 for assistance. We also would appreciate any feedback or comments that you may have with the use of this manual, and will incorporate any suggestions in future versions.

Sincerely,



W. Dean Rupp, Ph.D.
Chairman, Yale Biological Safety Committee
The Occupational Health & Safety Biological Safety Office

1 Introduction

1.1 Emergency Phone Numbers and Office of Environmental Health and Safety Contacts

Emergency Telephone Numbers

Ambulance/Fire/Police	111
University Health Services	432-0123
Biological/Chemical/Radiological Emergencies	785-3555 (Monday - Friday, 8:30 AM to 5:00 PM, at other times call Campus Police at 111)

Office of Environmental Health and Safety Telephone Numbers

Office of Environmental Health and Safety	785-3550
Benjamin Fontes, Biosafety Officer	737-5009
Maryjo Lanzillotta, Biosafety Associate	737-2127
Deborah Ferry, Biosafety Assistant	737-2125
Linda Mouning, Administrative Assistant	737-2121

Office of Environmental Health and Safety Web Site

<http://www.yale.edu/oehs>

1.2 Responsibilities

1.2.1 Department Chairperson

The Department Chairperson bears overall responsibility for the implementation and maintenance of safe practices and procedures in the department. The Chairperson, especially in the case of large departments, may share this responsibility with a departmental biological safety committee and/ or a unit director.

1.2.2 Principal Investigator

The principal investigator has the responsibility and authority for assessing risks, establishing policies and procedures, training personnel and maintaining the facility and equipment.

The principal investigator is responsible for:

- ◆ Performing appropriate risk assessment of research projects. The level of detail should be dependent on the hazard associated with the organism under study (e.g., an assessment of risk associated with research on BL2 agents might reasonably be less detailed than a risk assessment of BL3 or unknown agents). Each evaluation should be completed before work is undertaken and the project should be reassessed periodically as new data is obtained. The assessment should include an analysis of the risks posed by the particular organism under investigation and of any specific research methods that may affect that risk (e.g., procedures requiring highly concentrated amounts of virus or inoculation of laboratory animals). No human or animal pathogen should be studied without prior written approval of the Biological Safety Committee. The procedures for handling unclassified agents must also be reviewed by the Yale Biological Safety Committee and the Office of Environmental Health and Safety (OEHS), as well as the Yale Animal Resource Center (YARC) if work with animals is anticipated. The agents must be registered and information about these agents must be provided to the Office of Environmental Health and Safety.
- ◆ The application of appropriate safety practices and procedures within their laboratories and instructing students and staff of potential hazards.
- ◆ Approving research personnel to work in the laboratory and documenting that personnel are competent to conduct the work.
- ◆ Developing policies governing the operation of the laboratory and implementing protocols to ensure safe operation.

- ◆ Maintaining a liaison with the Biosafety Office.
- ◆ Registering research work involving non-exempt recombinant DNA with the Biological Safety Committee. The principal investigator must complete the "Registration of Recombinant DNA Experiments" application. The application must have details of the nature of the proposed experiments and an assessment of the levels of physical and biological containment required for them as established by the NIH guidelines.

Below is a table outlining the registration, training, and inspection requirements for which the Principal Investigator is responsible.

1.2.2.1 Table of Principal Investigator Requirements

	Required Registration							Required Training					Required Inspection							
	Form 01 and annual update	rDNA	Biological Safety Committee	State of Connecticut	Yale Animal Resource Center	Yale Animal Care and Use Committee	Yale HIC, FDA, NIH and YNHH	CDC	USDA	Bloodborne Pathogens	Biosafety	Biosafety Level 3	YARC	Shipping & Transport of infectious Agents	Annual Biological/Chemical	Biosafety	CT Dept. of Public Health	CDC Select Agent	USDA	Infection Control (Patient Areas)
If using items below requirements indicated with an “X” must followed																				
Human Materials and Human Origin Cell Lines	X		X						X					X						
Recombinant DNA – exempt	X									X				X						
Recombinant DNA – non-exempt	X	X	X							X				X	X					
Infectious Agents – BL2	X		X	X						X				X	X	X				
Infectious Agents – BL2+ and BL3	X		X	X							X			X	X	X				
Animal Use	X				X	X						X		X						
Animal Use with BL2 agent	X		X		X	X				X		X		X	X					
Animal Use with BL2+ or BL3 agent	X		X		X	X				X	X	X		X	X					
Human Gene Therapy	X	X	X				X		X	X				X						X
Select Agents – See list in Section 12	X		X					X		X				X	X		X			
USDA Regulated Agents	X		X					X						X	X			X		
Shipping Infectious Materials													X							

1.2.3 Research Personnel

Research Personnel are responsible for:

- ◆ Complete requirements for approval to work in the laboratory and ensure that all work is conducted in compliance with Yale University, NIH, CDC, OSHA and other applicable guidelines. Follow the Yale University Biological Safety Manual except where superseded by the *BL3 Manual* or the *Bloodborne Pathogens Training Manual*.
- ◆ Learn the operating procedures for the laboratory, the potential hazards of the infectious agents in use and emergency procedures. Help maintain the facility in good working condition.
- ◆ Report to the Principal Investigator any medical restrictions, reportable illnesses, and any event that may be an exposure or result in the creation of a potential hazard. Report all irregular conditions.
- ◆ If inexperienced in handling human pathogens or tissue cultures, receive training and demonstrate proficiency in standard microbiological practices from the Principal Investigator.
- ◆ Complete any medical surveillance requirements.
- ◆ Perform assigned responsibilities. The operation of the facility is the responsibility of the users; therefore a number of tasks must be assigned. These tasks are as follows:
 - Training
 - Autoclaves and waste
 - Freezers
 - Cleaning
 - Vacuum trap and filter maintenance
 - Maintenance of supplies, including personnel protective equipment
 - Security of infectious agents; i.e. store infectious agents in a locked freezer in a locked laboratory

1.2.4 Office of Environmental Health and Safety

The Office of Environmental Health and Safety:

- ◆ Provides consultation on operation of the laboratory to ensure compliance with CDC, NIH, OSHA and state criteria.
- ◆ Provides information on regulations that apply to the laboratory.
- ◆ Advises on safe methods for new procedures, and provides advice in the event of large or high hazard biohazardous material spills.

An annual laboratory inspection program is also in place to ensure continued compliance with safety regulations.

The Biological Safety Officer (BSO) is responsible for the implementation of policy guidelines recommended by the Yale Biological Safety Committee (YBSC). The BSO identifies potential problem areas and suggests to the YBSC safety objectives to be achieved. In addition, the BSO is also the institutional biological safety officer for recombinant DNA research. Some of the specific biological safety services provided by the Occupational Health and Safety section of the Office of Environmental Health and Safety include:

- ◆ Evaluation and inspection of laboratory facilities for work with infectious agents and other hazardous biological agents;
- ◆ Investigation of laboratory accidents;
- ◆ Periodic updates of rDNA experiments to ensure compliance with the NIH *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*;
- ◆ Maintenance of training records for compliance with federal, state and University requirements;
- ◆ Consultation to members of the Yale community in matters related to biological safety.

- ◆ Identification and updating of areas of known and potential biohazard at Yale University on a regular basis.
- ◆ Dissemination of information for safety in biological research through periodic newsletters, demonstrations or special training courses as necessary.

The Office of Environmental Health and Safety also provides new personnel training.

The Office of Environmental Health and Safety, Occupational Health and Safety Section, Biosafety Office will be referred to as the Biosafety Office throughout this document.

1.2.5 Yale Biological Safety Committee

The Yale Biological Safety Committee (the Committee) shall serve as the Institutional Biohazards Committee (IBC) as defined in the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. As such, the Committee shall review applications for research involving recombinant DNA to determine whether the facilities, procedures, and practices meet the standards required by the University and the NIH. It shall, in addition, have the responsibility to certify annually to the NIH that such facilities, procedures, and practices, and the training and expertise of personnel meet NIH standards. Meetings called for the purpose of such review and certification may be open to the public. Minutes of these meetings shall be kept and made available for public inspection.

The committee's responsibilities include:

- ◆ Registering laboratories and approving containment and procedures to be used.
- ◆ Advising facility users on policies related to biohazard containment.
- ◆ Updating laboratory registrations periodically.
- ◆ Determining the necessity for special medical monitoring.
- ◆ Advising Yale on the suspension of access privileges for staff found to be in violation of policies and procedures governing facility use.

The Committee shall advise the President, Provost and Director of the Office of Environmental Health and Safety (OEHS) on policy matters concerned with the protection of personnel from biohazardous agents including both infectious organisms and allergens that may be present in either laboratory materials or the environment. The Committee shall also recommend guidelines relating to procedures and facilities used at the University, including such matters as safety training and health surveillance.

The Committee shall offer its counsel to all University personnel regarding matters of biological safety. The President and Provost may ask the Committee to inform the community about developments in the general area of biological safety.

The Committee shall oversee the activities of the Biosafety Office in the sense that it shall:

- ◆ review its objectives and performance goals,
- ◆ monitor its progress in meeting those objectives and goals, and
- ◆ recommend changes in the organization and activities of the Biosafety Office that the committee may find desirable.

The Committee shall meet regularly with the Director of the OEHS and the Biological Safety Officer to receive progress reports and advise on specific safety issues as well as on general safety policy.

On matters of oversight that involve the evaluation of performance by the Biosafety Office, the Committee may, at the discretion of the chair, meet in executive session. In such cases the Biological Safety Officer and the Director of the OEHS shall be excused from participation and voting.

2 Biosafety Requirements

The following information describes the requirements for Yale researchers as defined by the Yale Biological Safety Committee and the Biosafety Office. It is the responsibility of each Principal Investigator to ensure the laboratory is in compliance.

2.1 Registration for the Use of Biological Materials

All Principal Investigators are required to complete and submit a “Form 01 Registration for the Use of Biological Materials” (Form 01). A copy of the form is in Appendix A. The Office of Environmental Health and Safety must maintain accurate information regarding the use of biological materials (e.g., microorganisms, cell lines, human materials, animals, and toxins) by University personnel. OEHS policy requires all Principal Investigators to submit accurate information annually and when there are changes during the year regarding the addition or deletion of biological materials, addition or deletion of employees or changes in room locations.

Please note that the Form 01 is reviewed for compliance with the annual training requirements specified by the Occupational Safety and Health Administration (OSHA), the NIH *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and the Connecticut Department of Public Health. The Biosafety Office will assist in updating information during the annual biological and chemical safety lab inspection.

The Form 01 is available in PDF format through the Office of Environmental Health and Safety’s World Wide Web site, <http://www.yale.edu/oehs>.

2.2 Human Pathogens

Registration with the State of Connecticut Department of Public Health and the Biosafety Office is required before the initiation of research with a human etiologic agent. The Biosafety Office will assist with the State registration process and any required updates.

The Yale Animal Care and Use Committee, Yale Animal Resources Center, and Biosafety Office must approve all experiments involving the introduction of infectious agents or potentially hazardous biological materials into animals prior to initiation.

All researchers working with etiologic agents (Risk Group 2 or higher) must receive training in both biosafety and the microbiological procedures that will be utilized for the experiment. Biosafety training sessions are provided through the Office of Environmental Health and Safety on a monthly basis (call 785-3550 or check OEHS web site for the next scheduled training date.) The Principal Investigator is responsible for ensuring that all researchers are trained in the appropriate procedures and techniques used in the laboratory.

Principal Investigators and/or Lab Supervisors must contact the Biosafety Office (785-3550) before initiating work with Risk Group 2 or Risk Group 3 agents to ensure appropriate registration. Risk Group 2 and higher etiologic agents are listed in Appendix B (*Classification of Human Etiologic Agents on the Basis of Hazard*). The Classification of Etiologic Agents is also available as part of the NIH *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. Copies of the NIH guidelines are available through the Biosafety Office or through the Internet at the following web address: <http://www4.od.nih.gov/oba/guidelines.html>. Call the Biosafety Office for assistance with agents that are not listed.

The Biosafety Office will periodically monitor your facility and procedures as well as answer any questions regarding biosafety.

The Biosafety Office must be contacted before:

- ◆ work with a new infectious agent is initiated

- ◆ changing the scope or location of existing work
- ◆ providing infectious agents to another investigator on or off campus
- ◆ arranging for visiting researchers to work in your laboratory.

Work with Risk Group 3 agents (Biosafety Level 3) requires additional registration. Contact the Biosafety Office for additional information. Work with Risk Group 4 agents (Biosafety Level 4) is not permitted at Yale University.

2.3 Recombinant DNA Experiments

Yale Biological Safety Committee approval is required prior to the initiation of most non-exempt recombinant DNA experiments. A brief description of non-exempt and exempt recombinant DNA experiments is described in Appendix E (“Recombinant DNA Registration Form”).

Principal Investigators and/or Lab Supervisors must contact the Biosafety Office to:

- ◆ register non-exempt recombinant DNA work
- ◆ update current registration if the scope of the work has changed
- ◆ ask any questions regarding recombinant DNA work.

The Recombinant DNA Registration form is in Appendix E, and is also available from the Biosafety Office or the OEHS web site. The NIH *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* is available from the Biosafety Office and the OEHS web site at www.yale.edu/oehs.

2.4 Human Gene Therapy (HGT)

Proposed clinical trials involving human gene transfer require registration and approval from both campus and federal agencies before initiation. The Yale University Institutional Biological Safety Committee requirements for human gene therapy protocols are detailed in “Guidelines for Human Gene Transfer Clinical Trials” in Appendix F.

2.5 Human Blood, Body Fluids, Tissue and Other Potentially Infectious Materials

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or certain body fluids (bloodborne pathogens.) The Bloodborne Pathogens Standard applies to all employers having employees that are “occupationally exposed” to human blood or other potentially infectious materials. An employee is considered occupationally exposed if there is “reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of an employee’s duties.” Other potentially infectious materials include:

- | | |
|--|---|
| ◆ Human cell or tissue cultures | ◆ Organ cultures |
| ◆ Any unfixed tissue or organ, other than intact skin, from a human being (living or dead) | ◆ HIV- or HBV- containing culture media or other solutions |
| ◆ Human body fluids, except urine, feces, saliva or tears unless visibly contaminated with blood | ◆ Blood, organs or other tissues from experimental animals infected with HIV or HBV or other bloodborne pathogens |

An individual is also considered occupationally exposed if they do not have direct contact with blood or other potentially infectious material, if the employee uses equipment that is used to process or store blood, other potentially infectious materials or bloodborne pathogens.

All occupationally exposed employees are required by OSHA to attend a Bloodborne Pathogens training session prior to beginning work and annually thereafter. There are additional requirements for research

laboratories and production facilities engaged in the culture, production, concentration and manipulation of HIV and HBV.

OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens such as:

- ◆ Human Immunodeficiency virus (HIV)
- ◆ Hepatitis B virus (HBV)
- ◆ Hepatitis D virus
- ◆ Hepatitis C virus
- ◆ *Plasmodium* species
- ◆ *Treponema* species
- ◆ *Babesia* species
- ◆ *Borrelia* species
- ◆ *Brucella* species
- ◆ *Leptospira* species
- ◆ *Francisella* species
- ◆ *Streptobacillus moniliformis*
- ◆ Colorado Tick Fever viruses
- ◆ Arboviruses
- ◆ *Spirillum minus*
- ◆ Creutzfeldt-Jakob virus
- ◆ Human T-lymphotropic Virus Type I
- ◆ Hemorrhagic Fever viruses

Consult the *Bloodborne Pathogen Training Manual for Clinical and Laboratory Personnel* for additional information on the exposure control plan, training requirements, work practices, housekeeping, engineering controls, personal protective equipment, signs/label requirements, Hepatitis B vaccination, emergency actions, exposure incident procedures, post-exposure evaluation and follow-up, and recordkeeping. The manual is available on the OEHS web site at www.yale.edu/oehs. Contact the Biosafety Office at 785-3550 for assistance with exposure determination and for training information.

2.6 Animals

All research experiments involving animals must be conducted in accordance with the associated Yale Animal Care and Use Committee (YACUC) approved protocol. Animal research that involves a hazard (biological, radiological, or chemical) must have a Request to Use Hazardous Agents in Animals form filed with the associated protocol and be reflected in the approved YACUC protocol. Contact the YACUC at 785-5992 for additional information.

Once approved, the Yale Animal Resources Center (YARC) must be contacted prior to initiation to ensure that a safety protocol will be established. Researchers must meet with YARC personnel and the relevant safety personnel to outline standard operating procedures. An orientation to the assigned animal housing area will be provided. Call 785-2526 for additional information.

The Biosafety Office must approve work with human pathogens or recombinant DNA in animals (including transgenic animals) prior to initiation. Contact the Biosafety Office to initiate the approval process.

For additional information please contact YACUC at 785-5992 or YARC at 785-2526.

2.7 Biological Safety Cabinets (BSCs) and Other Laminar Flow Benches (LFBs)

The Clean Air Device Program was designed to ensure the health and safety of Yale employees, to protect research and clinical materials, as well as to prevent the environmental release of infectious materials.

The efficacy of BSCs and LFBs depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory.

All BSCs and LFBs at Yale must be placed on the Yale University certification/service contract and be certified at least annually. Any BSCs or LFBs not under the certification/service contract will be placed in storage status. The Office of Environmental Health and Safety will contact you to schedule the required annual certification.

Notify the Biosafety Office in advance when you plan to have BSCs or LFBs moved, placed in storage, transferred to a new owner, discarded, removed from Yale or obtained from another institution or

manufacturer. Contact the Biosafety Office if service or repairs are needed (e.g., replacing fluorescent lamps, switches, etc.) for your unit. BSCs must be professionally decontaminated with formaldehyde, by a certified technician, before a unit is relocated, stored, serviced (interior) or discarded.

The purchase of BSCs and other LFBs is coordinated through Yale University Purchasing Department and the Biosafety Office. The Biosafety Office reviews all BSC and LFB purchase requests. Contact the Biosafety Office for more information. Yale actively discourages the purchase and use of LFBs since air is blown across the work surface into the face and torso of the operator. The Biological Safety Committee and the Biosafety Office recognize that clean benches do not provide personnel or environmental protection from infectious or potentially infectious agents, allergens, chemicals or radioactive materials. If you are using a clean bench, contact the Biosafety Office for a review of your procedures.

For additional information on policies, procedures and use of BSCs consult the Clean Air Device Guide on the OEHS web site or contact the Biosafety Office at 785-3550.

2.8 Training

Successful completion of a range of biosafety training programs may be required prior to the initiation of your work at Yale University. Please review the following table for information on required training. You can access the OEHS Training schedule at <http://www.yale.edu/oehs> or by calling 785-3550. If you have any questions, please don't hesitate to contact a Biosafety representative at 785-3550.

Before initiating work involving:	You must satisfactorily complete the following training:	Training Options
Human blood, other potentially infectious materials, (including human cell lines) and bloodborne pathogens.	Bloodborne Pathogen Training: <ul style="list-style-type: none"> ◆ Required before initiation of work and ◆ At least annually thereafter. 	OEHS course or Web training or Self-study guide
Human or animal pathogens classified at BL2	Biosafety Training	OEHS course
Human or animal pathogens classified at BL3	Biosafety Level 3 Training	OEHS course (call Biosafety at 785-3550 to schedule a class)
Packaging, Shipping, Transporting, or Receiving Biohazards (Infectious agents, hazardous biological toxins, and human clinical specimens)	Shipping and Transport Training	OEHS course or Shipping self-study guide
Contact with patients in a clinical setting	Tuberculosis Training Infection Control Training Bloodborne Pathogens Training	OEHS course OEHS course See Above

3 Medical Surveillance Program

A medical surveillance program of University personnel engaged in biological research is conducted by Employee Health at 17 Hillhouse Avenue. The purpose of the program is to conduct periodic health assessments of employees, with attention devoted to factors or conditions associated with a particular biological agent a given individual might handle. For a particular employee, the medical surveillance program might call for any of a number of precautionary measures, including immunizations, a periodic physical examination and collection of a serum specimen.

The purpose of the medical surveillance program is to:

- ◆ recommend appropriate medical precautions to be followed, and
- ◆ do periodic reassessment of employees to determine if medical conditions associated with employment are prevalent and, if so, to undertake definitive measures to alleviate them.

The extent of medical surveillance for a given employee will vary greatly and be dependent upon:

- ◆ the nature of the research project in which involved,
- ◆ the biological agents to which directly or potentially exposed, and
- ◆ certain additional factors relating to the current or previous health status of the individual.

The principal investigator is to provide Employee Health with guidelines and descriptions of conditions that might have significance for personnel assigned to the laboratory.

Medical surveillance is provided without charge for any employee of Yale University whose job may result in potential exposure. For more information about this program contact the Department of Employee Health at (432-0071).

3.1 Tuberculosis (TB) Screening

Employees who face occupational exposure to Tuberculosis (TB) are enrolled in the University's Tuberculosis Exposure Control Plan. The Occupational Safety and Health Administration has identified workers from the following areas as potentially exposed:

- ◆ Healthcare facilities
- ◆ Long term care facilities
- ◆ Correctional facilities
- ◆ Homeless shelters
- ◆ Substance abuse treatment facilities
- ◆ Laboratories that may handle M. tuberculosis

New employees at risk must be tested for TB exposure by a tuberculin skin test (PPD) at time of hire (within 2 weeks of start date) to establish a baseline. All employees at risk must be PPD tested on an annual basis.

Employees who have been exposed to active TB cases must report the incident and undergo an initial baseline TB test at time of exposure and a follow up test at 3 months post exposure. Please contact Employee Health (432-0071) to arrange for PPD testing and for additional information regarding the University's TB Exposure Control Plan. Contact the Office of Environmental Health and Safety at 785-3550 for information on TB training.

3.2 Immunizations

In certain situations, personnel engaged in particular research activities would be immunized with appropriate vaccines, such as rabies, rubella and measles. Vaccines not commonly available will be

obtained, whenever possible, for those engaged in specific research with potential exposure to the agent in question.

Vaccine	Recommendations
Rabies Vaccine	Recommended for all personnel entering laboratories or animal facilities with rabies vaccination entrance requirements.
Hepatitis B Vaccine	Recommended for persons working with human blood, body fluids or tissues.
Vaccinia Vaccine	Prior to working with vaccinia, employees are required to receive a medical evaluation and counseling from Employee Health regarding vaccinia immunization. In cases where infected animals are not housed in filter-top cages or other primary containment devices, vaccination shall be required for room entry.
Arboviruses: Eastern and Western Equine Encephalitis Vaccines, Japanese Encephalitis Vaccine, Venezuelan Equine Encephalitis Vaccine, Yellow Fever Vaccine, Rift Valley Fever Vaccine	Prior to working with arboviruses, employees are required to receive a medical evaluation and counseling from Employee Health regarding possible immunization.
Lyme Disease Vaccine	Recommended for persons working with the Lyme Disease agent or vectors in research laboratories, with animals, or in fieldwork.
Other vaccines such as Salmonella typhi (Typhoid),	To be determined by the Employee Health Physician.

In some cases, appropriate follow-up serum samples will be collected at periodic intervals to measure vaccine-induced antibodies when indicated.

3.3 Medical Restrictions

3.3.1 Pregnancy

It is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Therefore, if pregnancy is possible while you are working in an infectious disease laboratory or laboratory engaged in work with infectious agents you should consult your Principal Investigator or supervisor. The Department of Employee Health is also available for questions regarding the potential harm from the biological agents present within your laboratory.

Women that are pregnant or become pregnant are encouraged to inform their supervisors or Principal Investigators and Employee Health. *Employees are urged to discuss exposure issues with their supervisors or principal investigators regarding associated risks of research being conducted and pregnancy.* Employee Health will give advice about precautions that might be necessary.

Employee Health is a resource for pregnant women to ask about any questions or concerns they may have regarding risks in their work environment. Employee Health may also act as a liaison between pregnant employees and their respective supervisors or principal investigators. Please contact Employee Health for a list of reproductive and fetal pathogens.

3.3.2 Reproductive Biological Hazards

The Employee Health Physician will offer confidential counseling to any woman or man of childbearing age working with reproductive pathogens or other potentially infectious materials. Reproductive biological hazards include, but are not limited to the following:

- ◆ Cytomegalovirus (CMV)
- ◆ Hepatitis B virus (HBV)
- ◆ Hepatitis E virus
- ◆ Human Immunodeficiency virus (HIV)
- ◆ Human parvovirus B19
- ◆ Rubella (German Measles)
- ◆ Lymphocytic Choriomeningitis virus
- ◆ Toxoplasma gondii (Toxoplasmosis)
- ◆ Listeria monocytogenes
- ◆ Varicella-zoster virus (chicken pox)

Whenever necessary, Employee Health along with the Biosafety Office will offer an opportunity to review work procedures in the lab to ensure that potential exposure is minimized. Consideration for reassignment to other tasks that don't involve exposure to the reproductive hazard (generally with actual pathogens, not necessarily for only other potentially infectious materials such as blood or body fluids) should be given. Also, Investigators actively working with reproductive hazards explain the risk assessment at time of hire.

3.3.3 Other Restrictions

Restrictions or recommendations will be made on an individual basis after discussion with the Employee Health Physician and the employee's personal medical doctor. Examples of conditions that might warrant special precautions are HIV infection, immunosuppressive conditions and drug therapy that suppresses the immune system. Therefore, if you are suffering from any of the above conditions, you must inform your physician and the Department of Employee Health about the situation.

3.4 Employee Serum Storage

Many infections do not result in an overt disease condition. Such infections are detected by development of antibodies to the agent in question. Therefore, Employee Health has established a program for persons engaged in BL3 research or working with non-human primates, which includes collection of pre-assignment serum. If an illness occurs which may be related to the agent the person is working with, additional serum samples will be collected.

4 Accidents

4.1 Emergency Procedures for Exposure Incidents

An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of an employee's duties. An employee who sustains a known or potential exposure incident must remove gloves and treat the affected area immediately by following the appropriate exposure incident response below.

4.1.1 Percutaneous Injury

Wash the affected area with antiseptic soap and warm water for 15 minutes.

4.1.2 Splash to Face

Flush affected area in eyewash for 15 minutes.

4.1.3 Aerosol Exposure

Hold your breath and immediately leave room. Remove Personal Protective Equipment (PPE) carefully. When removing PPE make sure to turn the exposed areas inward. Wash hands well with soap and water. Post spill sign on lab entry; lab should be evacuated for at least 30 minutes. PI must clear lab for re-entry.

For extensive BL2 contamination (i.e. centrifuge incident) or incidents involving BL2+ or BL3 agents, OEHS must be notified and will assume responsibility, in conjunction with the PI, to clear the laboratory for re-entry.

4.2 Reporting Incident

The employee must report the incident to his/her supervisor. The supervisor must complete a Department Head's Report of Injury form and a Health Service Report form documenting the route of exposure and the circumstances under which the incident occurred.

4.3 Medical Assistance

Employees are urged to call Employee Health (432-0071) after they have received proper available first aid at site of exposure. For certain exposures; such as non-human primate bites or scratches, tick or insect bites, or exposure to infectious agents; the employee will be advised to come in and be evaluated by Employee Health. In situations when Employee Health is not available or if more extensive treatment is required, the employee will be referred to the Urgent Visit department and given a follow up visit with Employee Health.

University Health Services will provide the post-exposure evaluation and follow-up at no cost to employees who experience "exposure incidents". The post-exposure monitoring periods are dependent on the type of exposure. This time period is related to the various incubation periods of the infectious agents.

Employees can obtain copies of their medical records by contacting University Health Services. These records are kept by the Medical Records Department, 17 Hillhouse Avenue, 432-0062. Yale University must retain medical records for your duration of employment plus 30 years.

4.4 Investigation of Laboratory Accidents

The Office of Environmental Health and Safety, in cooperation with the principal investigator and his or her staff, will conduct the necessary investigation of a laboratory accident. The goal of the investigation is the prevention of similar accidents as well as obtaining information concerning the circumstances and number of employees who have been exposed to the agent in question. In addition, the Office of

Environmental Health and Safety, in consultation with Employee Health might institute further steps to monitor the health of those who may have been exposed to the agent in question.

It should be emphasized that the reporting of accidents to the principal investigator or laboratory supervisor is the responsibility of the employee who has the accident. The principal investigator or the laboratory supervisor should then report the incident to Employee Health at University Health Services, 17 Hillhouse Avenue. Please also report incidents that did not result in an exposure (near miss) to OEHS. Evaluation of near misses can lead to alternative work practices and implementation of engineering controls to minimize future incidents.

Whenever an injury involves a sharp and human material (body fluid, tissue, cell line, etc.) the Biosafety Office must perform an investigation to determine if a safe sharps device is available to prevent future occurrences of the injury. If safe sharps devices are available they must be evaluated by the biosafety office in conjunction with the Group or Department. The incident must also be recorded on the University's Sharps Injury Log, maintained by the Worker's Compensation Office. The confidential log will include the type and brand of device involved in the incident; the Department or work area where the exposure incident occurred; and an explanation of how the incident occurred.

5 Risk Assessment and Risk Management

Responsibility for biosafety exists at all levels and is shared throughout the University. The President and Provost acknowledge the institution's role in providing a safe workplace and have given the Biological Safety Committee and Biosafety Office the authority to administer the campus biosafety program. The Biological Safety Committee establishes policies for the safe use of biohazards and for compliance with all applicable regulations. As an agent of the Committee, the Biosafety Office disseminates pertinent information; consults with faculty, staff, students and visitors; and monitors for non-compliance.

The researchers, clinicians, and technicians who perform work with biohazards are perhaps the most important component of the biosafety program, as they must incorporate the biosafety requirements and safety precautions into all facets of their work.

The Principal Investigator is ultimately responsible for safety within the laboratory. An integral part of this responsibility is to conduct a review of proposed work to identify potential hazards (risk assessment) and to adopt appropriate safety procedures before initiation of the experiments (risk management).

Certain experiments require advanced registration and Biological Safety Committee approval prior to initiation (See Section 2).

A risk assessment/risk management matrix has been prepared to illustrate key elements of the process (see below). Relevant sections providing additional details are indicated within the matrix. Information on the routes of exposure is included at the end of this section.

The five P's of risk assessment and risk management are:

- ◆ Pathogen – hazardous biological agent.
- ◆ Procedures – proposed experimental manipulations and safe work practices.
- ◆ Personnel – appropriate training and skills.
- ◆ Protective equipment – protective clothing and safety equipment.
- ◆ Place – laboratory design.

Consider the five P's in each facet of laboratory work. Properly conducted, risk assessment can help prevent exposure to biohazards and minimize the potential for laboratory acquired infection. Remember that prior planning prevents poor performance.

After reading this section and relevant sections of the Biological Safety Manual contact the Biosafety Office at 785-3550 for help applying the principles of risk assessment and risk management to experimental procedures.

5.1 Risk Assessment and Management Table

	Risk Assessment	Risk Management
Pathogen	<ul style="list-style-type: none"> ◆ Agent classification (See Appendix B) ◆ Routes of infection ◆ Infectious disease process ◆ Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors 	<ul style="list-style-type: none"> ◆ Registration – See Section 2 <ul style="list-style-type: none"> ▪ Biosafety Office ▪ Biological Safety Committee ▪ State of Connecticut - infectious agents ▪ USDA – restricted agents ▪ CDC – select agents ▪ FDA/NIH - human gene therapy
Procedures	<ul style="list-style-type: none"> ◆ Aerosol risk: sonicating, centrifuging, homogenizing, blending, shaking, etc. ◆ Percutaneous risk: needles, syringes, glass Pasteur pipettes, scalpels, cryostat blade/knife, etc. ◆ Splash/splatter risk: pipetting, microbial loop, etc. 	<ul style="list-style-type: none"> ◆ Written set of standard operating procedures (SOPs) with safety practices incorporated ◆ Adherence to basic biosafety principles ◆ Label labs, areas, and equipment housing BL2 or higher agents ◆ Conduct lab inspections to review practices and containment equipment ◆ Use trial experiments with non-infectious material to test new procedures/equipment
Personnel	<ul style="list-style-type: none"> ◆ Host immunity <ul style="list-style-type: none"> ▪ Neoplastic disease ▪ Infection ▪ Immunosuppressive therapy ▪ Age, race, sex, pregnancy ▪ Surgery (splenectomy, gastrectomy) ▪ Diabetes, Lupus ◆ Immunization ◆ Post-exposure prophylaxis ◆ Serum banking ◆ Attitude toward safety ◆ Comfort ◆ Open wounds, non-intact skin, eczema, dermatitis 	<ul style="list-style-type: none"> ◆ Safety training ◆ Prior work experience with biohazards ◆ Demonstrated proficiency with techniques ◆ Prompt reporting of all exposure incidents, near misses, as well as signs and symptoms of related disease to PI and Employee Health ◆ Investigation/review of incidents/spills, etc. to prevent future occurrence
Protective Equipment	<p>Protection (containment) for:</p> <ul style="list-style-type: none"> ◆ Aerosols – respirable size particles) <5µm ◆ Droplets/splatter ◆ Sharps 	<ul style="list-style-type: none"> ◆ Personal protective equipment (PPE): <ul style="list-style-type: none"> ▪ Respirators – HEPA, N-99, N-95, etc. ▪ Face (eye, nose, mouth) protection – mask and safety glasses, or chin length face shield ▪ Solid front gown or lab coat ▪ Gloves ◆ Biological safety cabinets ◆ Centrifuge safety buckets/rotors
Place – Laboratory facility	<ul style="list-style-type: none"> ◆ Risk group/biosafety level requirements ◆ Aerosol risk ◆ Restricted access 	<ul style="list-style-type: none"> ◆ Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open ◆ Labels ◆ Containment laboratory with directional airflow

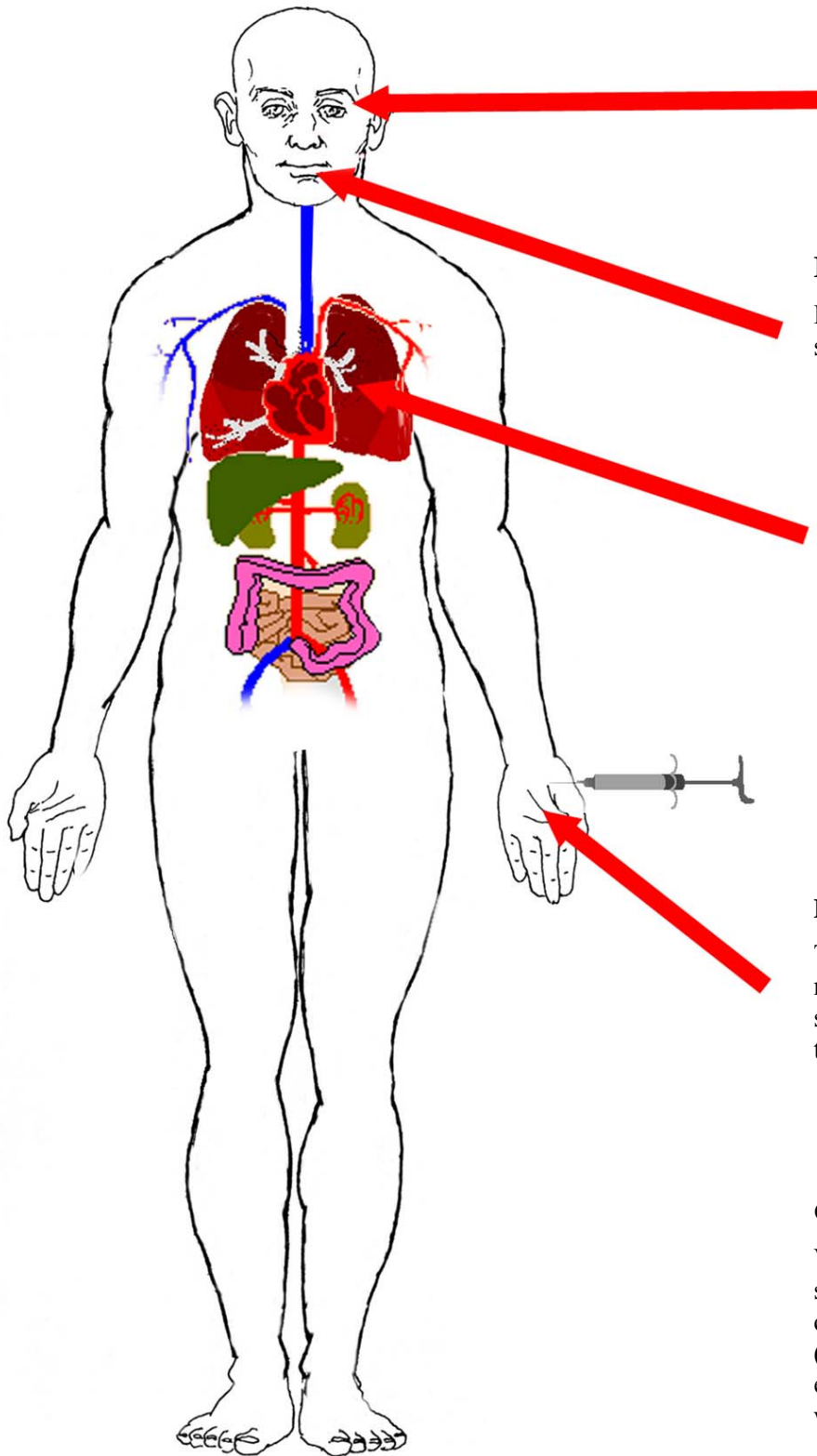
5.2 Routes of Exposures

In order for biological agents to cause disease, they must first enter or invade the body in sufficient numbers. Routes of entry include oral, respiratory, parenteral, mucous membrane and animal contacts (bites, scratches). Once inside the body, biohazards must meet other requirements to cause disease; they must colonize and establish in body cells, tissues and/or organs, overcome the body's natural defense mechanisms and mutate or adapt to body changes.

Other factors contribute to an individual's susceptibility to the disease process. These include age, immunological state, occupation, physical and geographic environment and predisposing conditions (such as alcoholism and other drug abuse, pregnancy and diseases such as diabetes).

It is difficult to determine a minimum infectious dose when discussing biohazards. The same dose of a pathogen may produce no disease symptoms in one individual but may cause serious or even fatal disease in another. There are microorganisms for which it is thought one organism entering the body is sufficient to invade and promote the disease process; the bacteria that causes tuberculosis is an example. For many pathogens, 10 to 100 or more organisms must enter the body to cause infection leading to disease. See the table below for additional information on routes of exposure or contact the Biosafety Office at 785-3550.

Routes of Transmission for Infectious Agents



Mucous Membranes:

Exposures to mucous membranes of the **eyes, nose and mouth** through splashes or splatters.

Ingestion:

Mouth pipetting, eating, drinking, smoking in the lab.

Inhalation:

Breathing in respirable sized aerosols ($<5\mu\text{m}$), centrifuge leaks, spills, pipetting, etc.



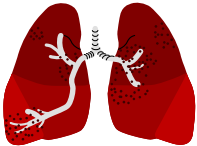


Percutaneous:

Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds abrasions, or eczema.

Contact (indirect transmission):

Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment handles) or by failure to wash hands after working

Protect for the Routes of Transmission

Route of Transmission	Protection
<p>Mucous Membranes</p>  <p>Through mucous membranes or the eyes nose or mouth (splash, splatter).</p>	<p>Face Protection:</p> <p>Full-face shield or safety glasses and surgical mask, Biosafety cabinet, protective shields, good microbiological practices.</p>
<p>Ingestion</p>  <p>Mouth pipetting, eating, drinking, smoking in the lab.</p>	<p>Good Microbiological Practices:</p> <p>Mechanical pipettors.</p>
<p>Inhalation</p>  <p>Breathing in respirable sized aerosols (<math><5\mu\text{m}</math>), centrifuge leaks, spills, pipetting, etc.</p>	<p>Biosafety Cabinet:</p> <p>Sealed rotors or canisters for centrifuges, safety containment equipment, HEPA filtered respirator, and good microbiological practices.</p>
<p>Percutaneous</p>  <p>Through intact or non-intact skin via needlestick, puncture with a contaminated sharp object, animal scratch, bite, through wounds, abrasions, or</p>	<p>Substitute plastic for glass:</p> <p>Use extreme precautions with sharps, dispose immediately in rigid leakproof needlebox, use animal restraints, cut resistant gloves, sleeve covers, water proof bandages, and double glove, good work practices.</p>
<p>Contact (indirect transmission)</p>  <p>Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment handles) or by failure to wash hands after working.</p>	<p>Decontamination of work surfaces and hand-washing:</p> <p>Good personal hygiene (avoid touching your face with glove or non-gloved hands), do not apply cosmetics within the laboratory.</p>

5.2.1 Routes of Transmission for Infectious Agents in the Laboratory

Route of Exposure	Protective Measures
Mucous Membranes. Exposure via the mucous membranes, eyes, nose, or mouth due to splash/splatter.	Achieve face protection by: <ul style="list-style-type: none"> ◆ wearing safety glasses and surgical mask or a full face shield ◆ working in a biosafety cabinet or behind a protective shield ◆ following good microbiological practices
Inhalation. Breathing in respirable aerosols (particles <5µm) due to centrifuge leaks, spills, or aerosol-generating procedures such as pipetting, homogenizing, etc.	Avoid exposure to aerosols by: <ul style="list-style-type: none"> ◆ working in a biosafety cabinet ◆ using sealed rotors or canisters when centrifuging ◆ following good microbiological practices
Ingestion. Exposure from mouth pipetting or eating, drinking or smoking in the laboratory.	Prevent exposure via ingestion by: <ul style="list-style-type: none"> ◆ never eating, drinking or smoking in the laboratory ◆ always using mechanical pipettors ◆ following good microbiological practices
Percutaneous. Exposure through intact or non-intact skin via needlestick, puncture with a contaminated sharp object, animal scratch or bite, through wounds, abrasions, eczema	Prevent percutaneous injuries by: <ul style="list-style-type: none"> ◆ substituting plastic for glass ◆ using extreme caution with sharps ◆ discarding sharps immediately into a rigid leakproof sharps container ◆ properly restraining animals ◆ wearing cut resistant gloves and sleeves ◆ covering non-intact skin with waterproof bandages and wearing double gloves
Contact (indirect exposure). Touching mucous membranes with hands that have been in contact with contaminated surfaces such as benches, phones, computers, etc. or hands that were not washed after working.	Prevent indirect exposure by: <ul style="list-style-type: none"> ◆ decontaminating work surfaces ◆ always washing hands when finished working or gloves have been compromised ◆ not touching face with gloves or non-gloved hands (good personal hygiene) ◆ not applying cosmetics within the laboratory

Whenever in the laboratory always adhere to the basic biosafety principles:

- ◆ do not eat, drink or smoke in the laboratory
- ◆ always wash hands when finished working or gloves have been compromised
- ◆ wear PPE within the laboratory. Be sure to remove PPE prior to leaving the laboratory
- ◆ never mouth pipette, always use mechanical pipettors
- ◆ use extreme caution when working with sharps
- ◆ contain aerosols by using appropriate equipment
- ◆ decontaminate work surfaces, spills and waste

5.3 Biosafety levels

The CDC and NIH have established biosafety levels for work with biohazardous materials in the publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). The publication provides combinations of microbiological practices, laboratory facilities, and safety equipment as well as their recommended use in four biosafety levels (BL) of laboratory operation with selected agents infectious to humans. Also included in the BMBL is a parallel set of biosafety levels for research involving small laboratory animals.

Below is a summary of practices, equipment and facility requirements for agents assigned to biosafety levels 1–4 (BL 1–4). Additional information on biosafety levels may be found in Appendix C as well as in the BMBL, which is available from the Biosafety Office and on the World Wide Web at <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

Only work at biosafety levels 1-3 is permitted at Yale University. No biosafety level 4 work is allowed at Yale University.

5.3.1 Summary of Recommended Biosafety Levels for Infectious Agents

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

Adapted from the Office of Health and Safety, Centers for Disease Control and Prevention

6 Signs and Labels

6.1 Wall Signs

6.1.1 Laboratory Safety Information Cards

A Laboratory Safety Information card must be completed and posted at the entryway to all laboratories to provide information on the materials handled inside the laboratory as well as the name and phone numbers of the principal investigator or other responsible person.

6.1.2 Biosafety Level

Entryways to research and clinical areas that handle BL2 materials, human blood or other potentially infectious materials must be posted with a BL2 biohazard sign that contains the universal biohazard symbol, the legend "Biohazard" and the term BL2. Entryways to research areas that handle BL3 material must be posted with a similar sign replacing the term BL2 with BL3.

6.2 Door Signs

HIV and HBV research laboratories and production facilities, laboratories working with certain infectious agents that require special provisions for entry (e.g. vaccination), and BL2+ and BL3 laboratories must have a biohazard door sign posted on all access doors.

The sign includes the international biohazard symbol, bears the legend "Biohazard", and identifies the name of the infectious agent, any special entrance requirements, and the name and phone numbers of the principal investigator or any other responsible persons. The following elements must be included on the door sign:



BIOHAZARD

(Name of infectious agent)

(Special entrance requirements)

(Name, telephone number of the principal investigator or other responsible person)

The door signs shall be fluorescent orange-red (or predominantly so) with lettering or symbols in a contrasting color.

6.3 Labels and Color-coding

Inside the facility, warning labels shall be affixed to containers of medical waste, refrigerators, freezers, incubators, and centrifuges containing BL2 or BL3 agents, human blood or "other potentially infectious material". Other equipment such as waterbaths, sonicators, and biological safety cabinets do not require a permanent biohazard label if decontaminated after each use. In these situations, a biohazard label should be temporarily posted on the equipment while in use with human blood, other potentially infectious materials, or an infectious agent.

Warning labels shall also be affixed to other containers used to store, transport or ship BL2 or BL3 agents, human blood or "other potentially infectious material". (Note: Shipping blood and "other

potentially infectious material” not suspected of harboring an infectious agent may not require the biohazard warning label, just the diagnostic specimen label). Labels required must have the international biohazard symbol and bear the legend "Biohazard" (see figure on next page).




BIOHAZARD

The labels shall be fluorescent orange-red (or predominantly so) with lettering or symbols in a contrasting color. Labels shall be affixed as close as feasible to the container by string, wire, adhesive, or any other method that prevents their loss or unintentional removal.

The use of warning labels may be waived if: (1) waste is placed in red bags or red containers; (2) containers of blood, blood components, or blood products are labeled as to their contents and have been released for transfusion or other clinical use; or (3) individual containers of blood or "other potentially infectious materials" are placed in a labeled secondary container during storage, transport, shipment or disposal.

6.4 Labeling Equipment Sent Out for Repair or Disposal

Contaminated and potentially contaminated equipment sent out for repair or disposal must be decontaminated as thoroughly as possible. Affix a tag to the equipment indicating when the equipment was decontaminated, what disinfectant was used, and the name of the person who performed the decontamination. Thorough decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. Decontaminate the equipment to the degree possible (flushing lines or wiping down the exterior) and affix a label to the equipment before sending it out for repair. The label must indicate what portions of the equipment remain contaminated and include the biohazard symbol as well as the legend "Biohazard". The label must convey this information to all affected workers (service representatives, manufacturer, etc.). Equipment tags can be obtained from the Office of Environmental Health and Safety at 737-2121. A sample of this tag appears below.

BIOSAFETY NOTICE		
This equipment's exterior and interior surfaces were decontaminated, and are free of any Biological Hazards. This notice does not apply to radiation or chemical hazards (if any).		
This equipment is released for (please circle one):		
Service/Repair	Relocation	Discard
Decontamination performed by: _____		
Chemical or disinfectant used: _____		
Date of decontamination: _____		
Location of equipment: _____		
Lab telephone number: _____		
Note: The following areas _____ of this equipment remain contaminated and a biohazard warning label has been attached near the contaminated area.		
Additional forms are available through the Office of Environmental Health and Safety		
	Yale University Office of Environmental Health and Safety 785-3550	

7 Laboratory Practices

In this section, an attempt has been made to provide information regarding hazards involved with certain laboratory practices and methods for preventing them. Prevention is an important element to biohazard control, and it is recommended that anyone working in a laboratory read this section carefully.

7.1 Human Factors and Attitudes in Relation to Laboratory Accidents

For the purpose of safety, an attitude can be defined as an accumulation of information and experience that predisposes an individual to certain behavior. Human factors and attitudes result in tendencies on the part of the individual to react in a positive or negative fashion to a situation, a person or an objective. Laboratory supervisors and Principal Investigators should understand the importance of attitudes and human factors in their own efforts to control biohazards in their laboratory. Some observations that may be of help to supervisors are listed below:

- ◆ The lack of accident perception ability is often a significant factor in laboratory accidents.
- ◆ Inflexibility of work habits, that tend to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.
- ◆ Working at an abnormal rate of speed is a significant causal factor.
- ◆ Intentional violations of regulations are a frequent cause of accidents. This is termed excessive risk taking.
- ◆ The performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
- ◆ Working when one is very tired is more likely to create a higher potential for accidents.
- ◆ Working at a well-organized and uncrowded laboratory bench will help in the prevention of lab accidents.

Each employee working with biohazardous agents must be consistently aware of the importance of the proper attitude in preventing accidents in the laboratory.

7.2 Biosafety Level 1

- ◆ Keep laboratory door closed when experiments are in progress.
- ◆ Use procedures that minimize aerosols.
- ◆ Do not smoke, eat, drink or store food in BL1 areas.
- ◆ Wear laboratory gowns or coats when appropriate.
- ◆ Do not mouth pipette. Use mechanical pipetting devices.
- ◆ Avoid using hypodermic needles.
- ◆ Wash hands after completing experimental procedures and before leaving laboratory.
- ◆ Disinfect work surfaces daily and immediately after a spill.
- ◆ Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
- ◆ For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
- ◆ Control insect and rodent infestations.
- ◆ Keep areas neat and clean.

7.3 Biosafety Level 2

- ◆ Keep laboratory door closed.
- ◆ Post a universal biohazard label on equipment where infectious agents are used/stored.
- ◆ Allow only persons informed of the research to enter BL2 areas.

- ◆ Keep animals not used in BL2 experiment out of the laboratory.
- ◆ Do not smoke, eat, drink, store food or apply cosmetics in BL2 areas.
- ◆ Wear PPE (laboratory gowns or coats, gloves and full-face protection) when appropriate; do not wear PPE outside of the laboratory.
- ◆ Wash hands after removing PPE as well as before leaving laboratory.
- ◆ Change PPE when soiled or compromised.
- ◆ Do not mouth pipette. Use mechanical pipetting devices.
- ◆ Use procedures that minimize aerosol formation.
- ◆ Avoid using hypodermic needles.
- ◆ Substitute plastic for glass where feasible.
- ◆ Use biological safety cabinets to contain aerosol-producing equipment.
- ◆ Wash hands after completing experimental procedures and before leaving laboratory.
- ◆ Disinfect work surfaces daily and immediately after a spill.
- ◆ Maintain a biological spill kit within the laboratory.
- ◆ Report spills, accidents, near misses and disease symptoms related to laboratory acquired infection to the PI.
- ◆ Ensure that all biomedical waste containers are labeled with the biohazard symbol.
- ◆ Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
- ◆ For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
- ◆ Control insect and rodent infestations.
- ◆ Keep areas neat and clean.

7.4 Biosafety Level 2+

Biosafety level 2+ (BL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BL2 procedures. Generally, BL3 practices are mandated in a space designed for BL2 work. It is preferred that the BL2 laboratory be self contained with all equipment required for the experiment located within the laboratory. A biohazard door sign listing the agent in use, emergency contact, and entry requirements is posted on the door while BL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment has been decontaminated, the sign is removed and the laboratory is returned to standard BL2 or BL1 use.

All manipulations of BL2+ material are conducted in a class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures.

Additional requirements for work at BL2+ are listed in Appendix D on page XXV. Please consult the Biosafety Office prior to initiating any work at BL2+.

7.5 Cell Culture

- ◆ Wear long sleeved gowns with knit cuffs and long gloves when working in the biosafety cabinet.
- ◆ Glassware and other contaminated items should be disinfected or autoclaved before washing, reuse or disposal.
- ◆ Glassware should be thoroughly cleaned and rinsed, by washing repeatedly with tap water and distilled water.
- ◆ Cell culture wastes must be decontaminated.
- ◆ Maintain a clean lab coat reserved solely for cell culture work.
- ◆ Avoid talking during culture manipulations as aerosols may be drawn into the work area.
- ◆ Place pipettes on a rack to avoid disrupting airflow when removed.

- ◆ Keep open tubes parallel to the airflow.
- ◆ After transferring inoculum always recap vials.
- ◆ Do not place tubes on work surface.
- ◆ Discard empty tubes immediately.
- ◆ Work with one specimen at a time; recap before going to the next.
- ◆ Autoclave verification should be performed routinely.

If a problem with contamination develops please refer to Appendix G of this manual and call the Biosafety Office for further assistance.

7.6 Transport of Biohazards on Campus (between labs or buildings):

- ◆ Must have two leakproof containers, including the following:
 - a sealed primary container
 - a sealed secondary container
 - absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
 - a biohazard sticker on the outside of the secondary container with agent name
 - lab address and phone number on the outside of the secondary container
- ◆ Utilize plastic containers whenever feasible. Avoid glass.
- ◆ Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.
- ◆ If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.
- ◆ Decontaminated the outside of the primary container before placing into the secondary container.
- ◆ Decontaminate the secondary container before leaving the laboratory.

7.7 Basic Microbiological Practices

Culture Plates, Tubes and Bottles

In the absence of definite accidents or obvious spillage, it is not certain that the opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means. Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a biological safety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is re-suspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture of colony. Following use of inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop or needle. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water of syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. Vented plastic Petri dishes, where the lid touches the rim at only three points, are less likely to offer this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent dispersal. If plates are obviously wet, they should be opened in the biological safety cabinet.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long sleeved laboratory garment.

Dried, infectious culture material may also collect at or near the rim or neck of culture tubes/flasks and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened in a biological safety cabinet.

Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created. Aerosol creation should be prevented or minimized; opening of ampoules should be done in biological safety cabinets. When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a biological safety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then wrap the ampoule in disinfected wetted cotton, and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw the contents into a fresh container. Some researchers may desire to use commercially available ampoules prescored for easy opening. However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leakproof secondary containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel. The secondary container must be labeled with a biohazard label bearing the name of the infectious material.

Embryonated Eggs

Harvesting cultures from embryonated eggs is a hazardous procedure and leads to heavy surface contamination of the egg trays, shells, the environment, and the hands of the operator. It is essential that operations of this type be conducted in a biological safety cabinet. A suitable disinfectant should be at hand and used frequently.

7.8 Housekeeping

Well-defined housekeeping procedures and schedules are essential in reducing the risks associated with working with pathogenic agents and in protecting the integrity of the research program. This is particularly true in the laboratory operating under less than total containment concepts and in all areas used for the housing of animals, whether or not they have been intentionally infected. A well conceived and well executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminates in the event of inadvertent release of an etiologic agent. Less immediately evident are the benefits of establishing, among personnel of widely varying levels of education, some concepts of the nature and sources of contamination.

7.8.1 Objectives of Housekeeping

The objectives of housekeeping in the laboratory are to:

- ◆ Provide an orderly work area conducive to the accomplishment of the research program.
- ◆ Provide work areas devoid of physical hazards.
- ◆ Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems under study.
- ◆ Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.
- ◆ Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that may:

- ◆ Harbor microorganisms potentially a threat to the integrity of the biological systems under investigation.
- ◆ Enhance the survival of microorganisms inadvertently released in experimental procedures.
- ◆ Retard penetration of decontaminates.
- ◆ Be transferable from one area to another on clothing and shoes.
- ◆ With sufficient buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement
- ◆ Cause allergenic sensitization of personnel (e.g., to animal dander).

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other area in the laboratory has the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

7.8.2 Scope

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological materials are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential

biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The list on the next page outlines a portion of the terms requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the laboratory complex must be viewed.

- ◆ Aisles
- ◆ Bench Tops
- ◆ Biological Safety Cabinets
- ◆ Cold Rooms
- ◆ Deep Freezer Chests
- ◆ Dry Ice Chests
- ◆
- ◆ Eyewashes
- ◆ Floors
- ◆ Glassware
- ◆ Hallways
- ◆ Incubators
- ◆ Insect and Rodent Control
- ◆ Instruments
- ◆ Lab Entry and Exit Ways
- ◆ Lab Equipment Cleanup
- ◆ Refrigerators
- ◆ Supply Storage
- ◆ Waste Accumulations
- ◆ Work Surfaces

7.8.3 Assignment of Responsibilities

Housekeeping in the laboratory is one avenue that leads to safely accomplishing the research program. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. The supervisor should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by a contracted cleanup crew; delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements; and the location of concentrated biological preparations, as well as contaminated equipment used in their preparation and application, will be known.

8 Personal Protective Equipment (PPE)

Multidisciplinary research conducted in Yale University laboratories requires that personal protective equipment (protective clothing and safety apparatus/equipment) be used to protect the researcher from contact with infectious, toxic and corrosive agents, excessive heat, cold, fire and other physical hazards. Suitable Personal Protective Equipment (PPE) also protects the experiment from contamination. The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research operations and levels of risk associated with the research. While PPE is an important component of any biological safety program, PPE is used with the understanding that PPE serves as a second line of defense. Good laboratory techniques, procedures and appropriate laboratory equipment are the primary barriers against potential exposure to hazardous agents.

For additional information you are urged to consult the Biosafety Office. In the event the Biosafety Office does not have a listing of the kind of protective devices you are seeking, efforts will be made to acquire the information needed.

8.1 Laboratory Clothing

A commonly used PPE item within the laboratory is special clothing. Both reusable and disposable clothing is available. Whichever is used, it must be durable, designed to provide protection and prevent exposure of the skin to harmful agents, as well as be compatible with the methods of decontamination employed.

Laboratory clothing serves to protect the wearer, the experiment, and environment against contamination. If proper precautions are not taken, contaminated clothing may carry infectious materials outside the laboratory and into other work areas, cafeterias, or the home. Infectious agents can remain viable on cotton and wool fabrics and be disseminated from these fabrics.

Some additional points:

- ◆ Overt exposure to agents at all level of risk should be followed by immediate decontamination of the PPE and change into clean PPE to protect the worker, the experiments and the environment.
- ◆ Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.
- ◆ PPE worn within the laboratory should not be worn outside the facility to the library, cafeteria, or other places accessible to the public.
- ◆ Personnel should be encouraged to use disposable facial tissues instead of personal handkerchiefs.
- ◆ PPE should be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.
- ◆ All PPE should be decontaminated before being sent to the laundry or discarded. Treat contaminated areas of PPE with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
- ◆ Do not take PPE home to launder; select a laundry service that follows universal precautions.
- ◆ Change PPE as soon as feasible whenever it is compromised, soiled or torn.
- ◆ Wear appropriate sizes and keep an adequate supply of PPE available in the laboratory.
- ◆ Wash hands whenever PPE is removed.
- ◆ Do not touch door handles, elevator buttons, telephones, computers or other clean surfaces or items with gloved hands.
- ◆ Wear closed-toe shoes and long pants to guard against skin contamination or chemical exposure. Do not wear sandals or shorts in the laboratory.

8.1.1 Gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. Quality assurance is an important consideration.

No one glove can be expected to be satisfactory for all intended uses. Gloves may be fabricated of cloth, leather, natural and synthetic rubbers, or plastics. New formulations of synthetic rubber and plastic continue to be developed as research makes varied and changing demands on the protective capabilities of gloves. Changing applications lead to improved capabilities of impermeability, strength, flexibility, tactile sense and control. Within even the modest laboratory, the glove applications may be such that no less than four or five types of protective gloves need to be stocked and used.

Disposable (single use) gloves provide a barrier between infectious agents and the skin. Glove use is a basic precept of preventing infectious agent transmission. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common.

Gloves shall be removed and hands washed before exiting the laboratory. Use the one glove method, or an appropriate secondary container, when transporting materials through common use areas.

The Office of Environmental Health and Safety (OEHS) can provide information on gloves needed for various tasks, such as working with animals, dry ice, heat, acids, etc. Consult OEHS with details of your work to receive further information about the type and availability of gloves that will best meet your requirements.

Considerations for the selection and use of gloves:

- ◆ Gloves are not 100% leakproof; change gloves periodically and when soiled and always wash hands after removing gloves or other PPE.
- ◆ Gloves will not prevent needle sticks or other puncture injuries.
- ◆ Check gloves for visible tears before use.
- ◆ Avoid wetting examination gloves as water or disinfectants will encourage wicking and leaking
- ◆ Do not reuse examination gloves; discard contaminated gloves in a biohazard bag immediately after use.
- ◆ Double glove or use household utility gloves when cleaning spills. Household utility gloves may be decontaminated and reused (replace when compromised.)

8.1.2 Procedure for Removing Gloves

Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

When removing PPE, remove lab coat or solid front gown first, then remove gloves (aseptically), remove face protection last to avoid touching your face with contaminated hands. If wearing double gloves, remove outer gloves before removing lab coat or solid front gown.

8.1.3 Shoes

Shoes worn in the laboratory must be closed-toe. Protective shoes are required for certain work activities. When working with infectious agents it is advisable to wear shoe covers, which can be decontaminated (autoclaved) before disposal, over street shoes. For work in tissue culture laboratories it may be necessary to change from street shoes to specific laboratory shoes for protection of cultures from contamination.

In certain animal facilities the Yale Animal Resources Center requires personnel to wear overshoes to protect the animals in containment areas. Similarly, people who work with animals and do cage washing are required to wear protective shoes. All personnel working under the Yale Animal Resources Center must follow these and other recommendations of the Yale Animal Resources Center.

8.1.4 Gowns, Lab Coats, Jumpsuits, Aprons and Other Protective Clothing

Gowns, lab coats and jumpsuits protect the wearer's clothing and skin from contamination. As with all PPE, the type of clothing needed depends on the task being performed and the degree of exposure anticipated.

Solid front wrap-around clothing offers better protection than pull-over type clothing or clothing with front closures. Lab coats are not 100% leakproof; change PPE when soiled, and always wash your hands after removing any PPE. Lab coats or other protective clothing will not prevent needle sticks or other punctures. Spills and splashes occur most often in the chest or lap area. The contaminated surface must be touched during removal of a front closing jacket or lab coat. The contaminated portion often ends up in the wearer's face during removal of pullover clothing. Many workers prefer not to button up front closing jackets, which leaves street clothing exposed. If front closing jackets must be worn, strict measures shall be implemented to assure the clothing is closed at all times when performing procedures or tasks that may cause exposure.

Long sleeved garments with snug fitting cuffs are preferred over open or short sleeves. Snug fitting cuffs prevent splashes, splatters and aerosols from making contact with exposed skin on the lower arms. Longer single-use gloves can be pulled over snug fitting cuffs to seal out any infectious materials.

Plastic, vinyl or rubber aprons are usually worn over other protective clothing when extra protection is desired. Aprons are necessary for protection against liquids spilling or splashing on clothing. It is recommended that appropriate aprons be worn to protect against the potential harmful effects of liquid waste. Aprons may also be used to provide protection from steam and hot water in locations such as animal handling facilities, autoclave rooms and laboratory glasswashing rooms.

8.1.5 Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid, to splash on the head, face and eyes or contact lenses. A variety of face shields, head covers/hoods, protective goggles, and lenses are available from safety supply houses. The selection is dependent upon materials of construction, fit, comfort, and compatibility with the work and the overall facial area requiring protection.

Some of the considerations for selection and use of face and eye protection are indicated below:

- ◆ Face shields and hoods protect the face and the neck from flying particles and sprays of hazardous material; however, they do not provide basic eye protection against impacting objects.
- ◆ Shields should cover the entire face, permit tilting back to clean the face if desired, and be easily removed in the event of an accident.
- ◆ If an eye hazard exists in a particular operation or experiment, the soundest safety policy would be to require that eye or face protection, or both, be worn at all times by all persons entering or working in the laboratory.

- ◆ Contact lenses do not provide eye protection. It is recommended that contact lenses not be worn when working around chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses are worn, eye protection, such as tight fitting goggles, must be worn.

8.2 Respiratory Protection

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to ten organisms, when inhaled, may cause infection. Particles with an effective aerodynamic diameter of between 0.5 and 5.0 μm (the respirable fraction) are most effective at penetration and retention in the deep pulmonary spaces. Particles larger than 5 micrometers are generally trapped in the upper respiratory tract and eventually cleared or swallowed.

Engineering controls, such as the use of biological safety cabinets, should be always be considered as a first line of defense against respiratory infection when working with infectious organisms. Respirators should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.

Respirators vary in design, application, and protective capability. Respirators can be placed into two categories:

- ◆ air purifying
- ◆ supplied air

By far, the most commonly used respirators in laboratories are air purifying respirators. These protect by purifying the existing breathing air through a filter (for particulates) or cartridge (for gases and vapors). Standard air purifying respirators at Yale are $\frac{1}{2}$ mask, full face, or powered air purifying respirators (PAPR). These rely on the proper cartridge selection to filter out the contaminant. Dust masks that have been approved by NIOSH are also considered to be air purifying respirators. These are ranked by their filtering efficiencies and by whether they can be used in an environment containing oil aerosols. Approved dust masks will have one of the following designations – N95, N99, N100, R95, R99, R100, P95, P99, or P100. Proper selection of cartridges and respirators is very important and should not be made without input from the Office of Environmental Health and Safety. New regulations concerning respirators require initial and annual training and fit-testing, and well as medical surveillance of all respirator wearers. Please make sure that the Office of Environmental Health and Safety is notified whenever the use of a respirator is being considered. The Respirator Administrator in OEHS can assist in evaluating the procedure, selecting the proper respirator, and provide the required training and fit testing. The Employee Health Office must also be notified so that medical surveillance and clearance can be issued prior to wearing the respirator.

A copy of Yale University's Respiratory Protection Program is available at www.yale.edu/oehs.

8.3 Selection of PPE

Use the following PPE to minimize exposure via mucous membrane OR non-intact skin:

- ◆ For face protection, wear safety glasses and a mask, or a chin length face shield whenever splashing, splattering or droplets may be anticipated (any work with liquids on the open bench). An impact resistant face shield should be used when operating the autoclave. Impact resistant face shields will protect the user's face against splatters of hot liquids or broken glass fragments.
- ◆ Gloves and a lab coat are worn to protect the skin and clothing from contact with potentially infectious materials. Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists. Consider double gloving when working with cultures of infectious agents or handling

spills. Thicker household utility gloves can be worn for cleaning blood or BL2 spills. Utility gloves can be decontaminated and reused until the integrity of the glove is compromised. Temperature resistant gloves should be worn to protect hands from physical damage when working with very hot (autoclave) or cold (liquid nitrogen tank, -70°C freezer) materials.

- ◆ Sleeve covers are worn over lab coat and gown sleeves to provide protection to the sleeves and wrists from contamination when working in the biological safety cabinet. Disposable sleeve covers have tight fitting grips at both ends.
- ◆ Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin conditions or wounds. Avoid working with BL2, BL3 or other potentially infectious materials if non-intact skin cannot be adequately covered.
- ◆ Solid front gowns provide more protection to clothing and skin than lab coats. Solid front gowns are worn for high hazard infectious agent work. The tight fitting cuffs of the gown help to minimize wrist contamination.
- ◆ Impervious lab coats, gowns or aprons are worn when heavy contamination or soiling is likely.
- ◆ Head covers are worn to protect the hair and scalp from splatter or droplets when working with heavy contamination or when contact with the head is likely. When choosing a head cover make sure it is impervious to liquids (some head covers are not impervious).
- ◆ Shoe covers are worn over the shoes to protect shoes from contamination when working in heavily contaminated areas (such as large spills, crime scenes, morgues, cadaver dissection areas, surgical operation areas).
- ◆ Gowns, head and shoe covers also help keep contaminants from entering the sterile area in clean rooms and surgical suites.

Use the following PPE to minimize exposure via cuts, slices, or scratches:

Kevlar gloves and sleeves are cut resistant and will help guard against slices, scratches or cuts, but will not prevent direct puncture or needlestick injuries. Steel mesh gloves also protect against slices, cuts, and scratches but will not eliminate punctures. Neoprene and other abrasive resistant gloves are cut resistant, but significantly reduce dexterity.

Use the following PPE to minimize exposure via aerosols:

HEPA filtered respirators (air purifying or powered air purifying) are worn to prevent exposure to potentially infectious aerosols when cleaning spills of concentrated infectious material or responding to centrifuge incidents. Employees who wear a respirator must enroll in the Yale Respiratory Protection Program before using a respirator.

8.4 PPE Requirements Table

PPE	Biosafety Level 1	Biosafety Level 2	Biosafety Level 3
Gloves	Recommended to prevent skin or clothing contact with BL1 materials. Note: work that may involve radioactive materials or chemicals will require the use of a lab coat and gloves.	Required	Required
Lab Coat	Recommended to prevent skin or clothing contact with BL1 materials. Note: work that may involve radioactive materials or chemicals will require the use of a lab coat and gloves.	Required	Solid front protective clothing such as back fastening gown with tight fitting cuffs must be worn to protect street clothing and skin from contact with infectious agents.
Face Protection		Wear protective eyewear and surgical mask or chin length face shield whenever splashing, splattering or spraying is anticipated to prevent contact with mucous membranes of the eyes, nose and mouth. Researchers may choose to augment eye protection by performing experiments behind a protective splash shield.	Face protection is not required when performing all work inside a biological safety cabinet. However, if there is a potential for splashing, such as from a dropped container during transport, face protection must be worn.
Respiratory Protection			The use of respiratory protective equipment such as a powered air purifying respirator (PAPR) will be recommended or required by the Yale Biological Safety Committee and/or the Office of Environmental Health and Safety (OEHS) on a case by case basis. The use of PAPRs is required for response and cleanup of a BL3 spill. All those who may wear a respirator must be enrolled in the OEHS Respiratory Protection Program.
Other		Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons, and household rubber gloves will be recommended on a case by case basis. Generally, additional protective clothing is required whenever there is a high potential for splashing of potentially infectious material, such as organ harvesting or large spill response and clean up.	Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons, and household rubber gloves will be recommended on a case by case basis. Generally, additional protective clothing is required whenever there is a high potential for splashing of potentially infectious material, such as organ harvesting or large spill response and clean up.

9 Laboratory Equipment

9.1 Biological Safety Cabinets

The Office of Environmental Health and Safety has a program that monitors the performance of biological safety cabinets as well as horizontal and vertical laminar flow benches. Additional information on the program is found in the *Clean Air Device (Primary Containment Device) Program Guide*. The program conforms to guidelines established by the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) and the Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogens Standard.

Biological safety cabinets (BSCs), when used properly, provide a clean work environment for research or patient care activities. Biological safety cabinets offer personnel, product, and environmental protection. The BSC provides primary containment for infectious materials. The efficacy of BSCs depends upon the behavior of the operator and the orientation of the unit in the facility.

The BSC isolates biohazards from personnel by confining the biohazardous material in the unit. The BSC removes aerosolized biohazardous material by moving air through high efficiency particulate air (HEPA) filters. The intake air is filtered through a HEPA filter before entering the BSC work area. Exhaust air also passes through a HEPA filter. Aerosols generated in the work area of the BSC are contained within the BSC.

Operating Procedures for Class II Biological Safety Cabinet:

- ◆ If used, turn off UV light; turn on fluorescent light and blower.
- ◆ Disinfect all interior surfaces with 70% ethanol or suitable disinfectant.
- ◆ Place items required for procedure into cabinet; do not obstruct grills.
- ◆ Wait 2-3 minutes for contaminants to purge from work area.
- ◆ Keep materials at least 4 inches inside work area.
- ◆ Work should proceed from clean to contaminated areas.
- ◆ After procedure, allow cabinet to run 2-3 minutes before removing materials.
- ◆ Wipe down all work surfaces with 70% ethanol or suitable disinfectant.
- ◆ Turn off fluorescent light and blower if desired.

Many BSCs are equipped with germicidal ultraviolet (UV) lamps. Time of exposure, distance, presence of dust or debris and UV lamp intensity affect the germicidal effect of the UV lamp. The visible blue-violet glow of the UV lamp does not indicate there is germicidal effect. The UV lamp needs to be cleaned periodically to remove dust. UV lamps may damage eyes, skin, and laboratory equipment. UV lamps should be turned off while the room is occupied.

OEHS discourages the use of UV lamps due to the potential damage resulting from UV lamp use.

9.2 Procedures for Centrifugation

All centrifugation shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. If a small centrifuge is used and centrifuge safety cups are not available, the centrifuge should be operated in the biological safety cabinet.

Each person operating a centrifuge should be trained on proper operating procedures.

Keep a log book detailing operation records for centrifuges and rotors to assist in determining service requirements.

The following procedures for centrifugation are recommended:

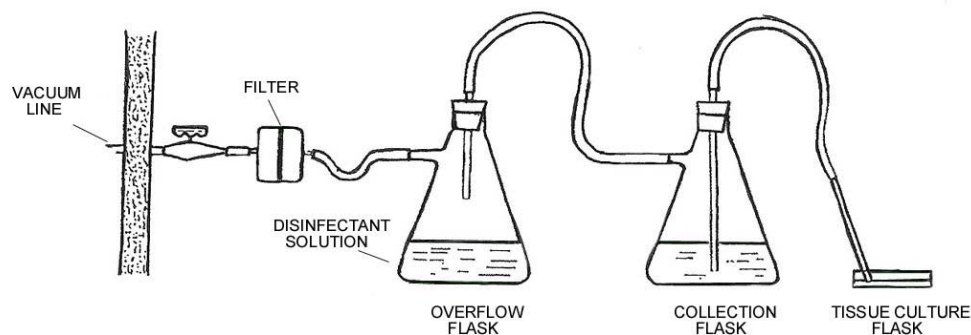
- ◆ Examine tubes and bottles for cracks or stress marks before using them.
- ◆ Fill and decant all centrifuge tubes and bottles within the biological safety cabinet. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- ◆ Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- ◆ Always cap tubes before spinning.
- ◆ Place all tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Correct rough walls caused by erosion or adhering of matter and remove debris from the rubber cushions.
- ◆ Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket.
- ◆ Never exceed safe rotor speed.
- ◆ Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- ◆ Wait five minutes after the run before opening the centrifuge. This will allow aerosols to settle in the event of a breakdown in containment.
- ◆ Decontaminate safety carriers or rotors and centrifuge interior after each use.
- ◆ Open safety buckets or rotors in a biological safety cabinet. If the rotor does not fit in the biological safety cabinet, use the fume hood.
- ◆ If construction of the centrifuge permits, the centrifuge chamber is to be connected to a vacuum pump with a HEPA filter installed between the centrifuge and the vacuum pump.

9.3 Vacuum Line Chemical Traps and Filters

Vacuum line chemical traps and filters prevent suction of infectious and non-infectious materials into the vacuum lines. Two techniques for devising chemical traps and filter systems are reprinted from the *Laboratory Safety Monograph* below. Contact the Biosafety Office for information regarding vacuum line filters.

Considerations and Limitations of Vacuum Line Chemical Traps and Filters:

- ◆ Add full strength chemical disinfectant to chemical trap flasks. Allow the aspirated fluids to complete the dilution. (For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard.)
- ◆ Vacuum line filters shall be examined and replaced if clogged or if liquid makes contact with the filter. Used filters shall be discarded in the medical waste stream.



9.4 Syringes and Needles

The hypodermic needle is a dangerous instrument. To lessen the chance of accidental injection, aerosol generation, or spills, the use of syringes should be avoided when alternate methods are available. For example, use a blunt needle or cannula on the syringe for oral or intranasal inoculations and never use a syringe and needle as a substitute for a pipette in making dilutions.

The following practices are recommended for hypodermic needles and syringes when used for parenteral injections:

- ◆ Use the syringe and needle in a biological safety cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.
- ◆ Examine glass syringes for chips and cracks, and needles for barbs and plugs. This should be done prior to sterilization before use. Use needle-locking syringes only, and be sure that the needle is locked securely into the barrel. Replace glass syringes with plastic disposable syringes whenever possible.
- ◆ Whenever possible use safer needle systems.
- ◆ Wear latex gloves for all manipulations with needles and syringes.
- ◆ Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- ◆ Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with an appropriate disinfectant, or into a small bottle of sterile cotton.
- ◆ Do not use the syringe to forcefully expel a stream of infectious fluid into an open vial for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the syringe is held below the surface of the fluid in the tube.
- ◆ If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in the transfer of infectious material to the fingers.
- ◆ When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pledget may be used and immediately discarded into a biohazard bag.
- ◆ When inoculating animals, position the hand that is holding the animal “behind” the needle or use a pair of forceps to hold the animal in order to avoid puncture wounds.
- ◆ Be sure the animal is properly restrained prior to the inoculation and be on the alert for any unexpected movements of the animal.
- ◆ Before and after injection of an animal, swab the injection site with an appropriate antiseptic.
- ◆ Discard syringes into a beige sharps container. DO NOT bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable, use a one handed method. DO NOT discard syringes into a red bucket or biohazard bag.

9.5 Pipettes

The following is excerpted from *Laboratory Safety, Principles and Practices* 2nd Ed., ASM Press.

- ◆ Never suction or pipette by mouth; always use some type of pipetting aid when pipetting infectious materials. Preferably, all activities should be confined to a biosafety cabinet.
- ◆ Mouth pipetting should be prohibited even with mouth pipetting devices that use an hydrophobic membrane filter that does not require fingers to touch the mouthpiece. This reusable pipetting device requires storage on the bench or other location between usage, which can result in contamination on the end piece that inserts into the mouth.
- ◆ Pipetting of toxic chemicals should be performed in a chemical fume hood.
- ◆ Infectious or toxic materials should never be forcefully expelled from a pipette. Mark-to-mark pipettes are preferable to other types because they do not require expulsion of the last drop.

- ◆ Infectious or toxic fluids should never be mixed by bubbling air from a pipette through the fluid.
- ◆ Infectious or toxic fluids should never be mixed by alternate suction and expulsion through a pipette.
- ◆ Discharge from a pipette should be as close as possible to the fluid or agar level, and the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.
- ◆ Pipettes used for transferring infectious or toxic materials should always be plugged with cotton, even when safety pipetting aids are used.
- ◆ Avoid accidentally dropping infectious or toxic material from the pipette onto the work surface. Place a disinfectant dampened towel or other absorbent material on the work surface, and autoclave before discard or reuse. Plastic backed bench paper is suitable for this purpose.
- ◆ Contaminated pipettes should be placed horizontally into a pan or tray containing enough suitable disinfectant, such as hypochlorite, to allow complete immersion of the pipettes. Pipettes should not be placed vertically in a cylinder that, because of its height, must be placed on the floor outside the biosafety cabinet. Removing contaminated pipettes from the biosafety cabinet and placing them vertically in a cylinder provides opportunity for dripping from the pipette onto the floor, or the rim of the cylinder, thereby creating an aerosol, and the top of the pipettes often protrude above the level of disinfectant.
- ◆ Place discard pans for used pipettes within the biosafety cabinet.
- ◆ After suitable contact time, excess disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together, and replaced by a clean pan with fresh disinfectant.

9.6 Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding or disrupting biohazardous materials. Even the use of a mortar and pestle can be a hazardous operation. Other devices that may produce aerosols are ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers.

Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Wherever sonicators are used in the cleaning process; such as in dishwashers, animal cage washers, etc.; all items should be sterilized prior to cleaning.

The laboratory practices generally required when using equipment that may generate aerosols with biohazardous materials are as follows:

- ◆ Operate blending, cell disruption, and grinding equipment in a biological safety cabinet;
- ◆ Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leakproof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline, or methylene blue solution is recommended prior to use;
- ◆ If the blender is used with infectious material place a towel moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use;
- ◆ Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break;
- ◆ Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product;
- ◆ Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud;
- ◆ Grinding of infected tissues or materials with any open device is best done within a biological safety cabinet.

9.7 Lyophilizing

Specimens shell-frozen in ampoules are dried on a vacuum manifold or in a chamber-type drier at low negative pressure. If the glass neck of the ampoule is sealed off while the ampoule is still under vacuum, it may cause implosion, either during the sealing or later when the evacuated ampoule is being opened. To avoid this, after drying is completed, and before sealing is done, bring the pressure within the ampoule back to normal by gradually introducing dry nitrogen, avoiding turbulent disturbance of the dry product.

The narrow or constricted neck of the ampoule is contaminated if the specimen is allowed to run down the wall of the neck during filling. Subsequently, when the ampoule is sealed with a torch, the dried material on the wall becomes charred or partially decomposed; residues of this material may adversely affect the dried material when it is reconstituted. To avoid this, a syringe with a long cannula or a Pasteur-type pipette should be used to fill the vial. Do not allow the delivery end of the cannula or pipette to touch the neck of the vial.

All ampoules used for freeze-drying of cultures, toxins, or other biohazardous material should be fabricated of Pyrex-type glass. This type of glass requires a high-temperature torch using an air-gas or oxygen-gas mixture for sealing. These hard glass ampoules are much less apt to form gas bubbles that burst inwardly during sealing under vacuum than the soft glass ampoules and are more resistant to breakage during handling and storage.

The filling of ampoules and vials with infectious specimens, the subsequent freeze-drying, and sealing or closing of ampoules and vials in the preparation of dry infectious specimens should be performed in a biological safety cabinet. The same is true for the preparation of ampoules and vials containing liquid specimens not subject to freeze-drying.

Safety precautions to be taken will depend on the agents, equipment, and containment available. Therefore, before initiating this procedure, the principal investigator should work out the protocol for each machine in consultation with the Biological Safety Officer. All persons using the procedure must then follow the protocol.

9.8 Microtome/Cryostat

Due to the very sharp blade and the nature of the materials used with the microtome/cryostat, training is essential in the use of the equipment and in the hazards of the materials used with the equipment. Users should be informed of the need to prevent cuts and scrapes as well as protect the eyes, nose, mouth and skin from exposure to the materials being used.

New personnel must be trained in the proper use and maintenance of the equipment, and demonstrate proficiency prior to use.

If using human tissue, microtome/cryostat users are required to attend Bloodborne Pathogens training. Fixatives take time to penetrate tissue; the fixatives may not inactivate pathogens deep in the tissue. Freezing and drying do not inactivate most pathogens, so, as with fixative use, the pathogens that may be present in the tissue should be considered capable of causing infection.

Microtome/cryostat users shall also attend Chemical Safety Laboratory Personnel training due to the fixatives and dyes used in histology.

When purchasing new units the available safety features should be taken into consideration prior to deciding on a manufacturer or model. Some available safety features are:

- ◆ Auto-decontamination cycle.
- ◆ Easy blade release for installing and changing blades.
- ◆ Retractable knife/blade to permit safe entry into chamber for cleaning, retrieving specimens, etc.
- ◆ Disposable blades.

Never retrieve samples, change blades, or clean equipment by hand with the blade in place; always use appropriate engineering controls (i.e. forceps, tweezers, dissecting probes, and small brushes).

Things to remember when using and maintaining microtomes/cryostats:

- ◆ Always keep hands away from blades.
- ◆ Use extreme caution when aligning blocks, the blocks may be close to the blades. If available, make sure block holder is in locked position when loading/aligning blocks.
- ◆ Use knife-edge protectors/guards. Do not leave knife-edges that may extend beyond microtome knife holder unprotected.
- ◆ Keep blocks wet when in the microtome to minimize airborne shavings during slicing.
- ◆ Use brushes to clean/brush equipment.
- ◆ Use engineering controls such as forceps when removing or changing the blade.
- ◆ Dislodge stuck blocks using mechanical means such as forceps and/or dissecting probes.
- ◆ Wear appropriate PPE such as a lab coat or gown, mask, safety glasses or goggles, surgical grade Kevlar gloves that provide dexterity and cut protection, and examination gloves to protect against biohazards.
- ◆ When changing blades, wear stainless steel mesh gloves to provide additional protection from cuts and scrapes.
- ◆ Avoid freezing propellants that are under pressure as they may cause splattering or droplets of infectious materials.
- ◆ Decontaminate equipment on a regular schedule using an appropriate disinfectant.
- ◆ Consider trimmings and sections of tissue as contaminated and discard in the appropriate waste stream.
- ◆ Do not move or transport microtome with knife in position.
- ◆ Do not leave knives out of containers when not in use.
- ◆ Do not leave motorized microtomes running unattended.

9.9 Miscellaneous Equipment (Waterbaths, Cold Storage, Shakers)

Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended. Sodium azide should not be used as a bacteriostatic. It creates a serious explosion hazard.

Deep freeze, liquid nitrogen, and dry ice chests as well as refrigerators should be checked, cleaned out periodically to remove any broken ampoules, tubes, etc. containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deep freezers should be properly labeled. Security measures should be commensurate with the hazards.

The degree of hazard represented by contaminated liquid nitrogen reservoirs will be largely dependent upon the infectious potential of the stored microorganisms, their stability in liquid nitrogen, and their ability to survive in the airborne state. Investigations suggest that storing tissue culture cell lines in containers other than sealed glass ampoules might result in potential inter-contamination among cell lines stored in a common liquid nitrogen repository.

Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as non-infectious until culture or other tests have proved their sterility.

Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

No person should work alone on an extremely hazardous operation.

10 Decontamination and Disposal Procedures

10.1 Decontamination Methods

Physical and chemical means of decontamination fall into three main categories: heat, liquid decontaminants, and vapors and gases.

10.1.1 Heat

The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121°C under pressure in the autoclave is the most convenient method of rapidly achieving sterility under ordinary circumstances. Dry heat at 160°C to 170°C for periods of two to four hours is suitable for destruction of viable agents on an impermeable non-organic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat for decontamination. Incineration serves as an efficient means of disposal for human and animal pathological wastes.

The hazards of handling hot solids and liquids are reasonably familiar. Laboratory personnel should be cautioned that steam under pressure could be a source of scalding jets if the equipment is misused. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too soon after treatment. This may cause a sudden and violent boiling of contents from the containers that can splash scalding liquids onto personnel handling the containers. See the autoclave safety poster in the Poster Section of this manual.

10.1.2 Liquid Decontaminants

In general, the liquid decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems.

There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, contact time, pH, the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in the effectiveness of decontamination. For this reason even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids and alkalis, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant the more likely it will possess undesirable characteristics such as corrosivity. None is equally useful or effective under all conditions for all infectious agents.

Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned to the task of making up use-concentrations from stock solutions must be informed of the potential hazards and trained in the safe procedures to follow and appropriate personal protective equipment to use as well as the toxicity associated with ocular, skin and respiratory exposure.

10.1.3 Vapors and Gases

A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in a closed system and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas

decontaminants are primarily useful in decontaminating biological safety cabinets and associated air-handling systems and air filters; bulky or stationary equipment that resists penetration by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; and rooms, buildings and associated air-handling systems.

Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas. In preparing use-dilutions and when applying them, personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary. Mutagenic potential has been attributed to ethylene oxide; toxic and hypersensitivity effects are well-documented for formaldehyde. An outside contractor performs formaldehyde decontaminations to minimize potential exposure to Yale University employees. Ethylene oxide use is very limited and is generally used in surgical and clinical areas.

Use of formaldehyde and ethylene oxide is monitored closely by the OEHS. Please contact the OHS (785-3550) for information regarding the exposure monitoring program.

10.2 Autoclave Procedure

Moist heat causes the denaturation of proteins at lower temperatures and shorter times than dry heat. One of the most effective physical decontamination controls is steam sterilization (autoclave) which generates moisture and high temperature pressurized steam within a sealed chamber. Autoclaves can sterilize all items that are heat stable. In gravity autoclaves, a cycle of 250°F (121°C) at 15 to 18 pounds per square inch (psi) of pressure for one hour may be required for decontamination. In the newer vacuum autoclaves, decontamination may require a cycle of 270°F (132°C) at 27 to 30 psi for 45 minutes.

A biological indicator should be used to verify proper autoclave operation. The Biosafety Office has indicators available for laboratory use; for more information contact the Biosafety Office at 785-3550.

Personal protection equipment (PPE) such as rubberized aprons, full-face shields and heat and liquid resistant gloves must be worn when operating autoclaves.

Position items in the autoclave to allow steam penetration into all items to be decontaminated. Materials in tightly sealed or stoppered containers may not be effectively decontaminated and may become dangerously pressurized causing injury when removed from the autoclave.

Items containing chemicals such as phenol or chloroform should not be placed in an autoclave.

See the autoclave safety poster in the Poster Section of this manual.

10.3 Characteristics of Chemical Decontaminants

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, or liquid concentrates. These may be added to water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperature are useful as space-penetrating decontaminants. Others become gases at elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways:

- ◆ Coagulation and denaturation of protein, or
- ◆ Lysis, or
- ◆ Binding to enzymes or inactivation of an essential enzyme by oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants may be altered substantially by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending on how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay system employed. Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms rather than failure of the decontaminant to act. If an item is placed in a liquid decontaminant, tiny bubbles are visible on the surface of the item. The area under the bubbles is dry and microorganisms in these dry areas will not be affected by the decontaminant. If there are spots of grease, rust or dirt on the item, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful. A decontaminant should have, and most do have, incorporated surface-active agents.

10.3.1 Properties of Some Common Decontaminants

Alcohol

Ethyl or isopropyl alcohol in a concentration of 70-85% by weight is often used; however, both lose effectiveness at concentrations below 50% and above 90%. Alcohols denature proteins and are somewhat slow in germicidal action. However, alcohols are effective decontaminants against lipid-containing viruses. A contact time of ten minutes is generally employed in efficacy tests with disinfectants. Due to the high evaporation rate of alcohols, repeated applications may be required to achieve the required ten-minute contact time for decontamination. Because of this, the OSHA Bloodborne Pathogens Standard does not recognize alcohol as an effective decontaminant for surfaces.

Isopropyl alcohol is generally more effective against vegetative bacteria; ethyl alcohol is a more virucidal agent.

Formaldehyde

Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration referred to as formalin, or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures, and the pungent, irritating odors make formaldehyde solutions difficult to use in the laboratory. Formaldehyde vapor generated from solution is an effective space decontaminant for buildings or rooms, but in the vapor state in the presence of water tends to polymerize on surfaces to form paraformaldehyde, which is persistent and unpleasant. Heating paraformaldehyde to depolymerize it can liberate formaldehyde gas. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than is the case in the vapor state.

Phenols

Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. Phenolic compounds are effective decontaminants against some viruses, fungi, and vegetative bacteria, including rickettsiae. Phenolics are not effective in ordinary use against bacterial spores.

Quaternary Ammonium Compounds or Quats

After 40 years of testing and use, there is still considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions will quickly lose effectiveness in the presence of proteins. Quats tend to clump microorganisms and are neutralized by anionic detergents such as soap. They have the advantages of being nontoxic, odorless, stable, non-staining, non-corrosive to metals, and inexpensive.

Chlorine

This halogen is a universal decontaminant active against many microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in the presence of protein. Free available chlorine is the active element. It is a strong oxidizing agent and corrosive to metals. Chlorine solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25%, or 52,500 ppm, available chlorine. If diluted 1 to 100, the resulting solution will contain 525 ppm of available chlorine, and, if a nonionic detergent is added in a concentration of about 0.7%, a very good decontaminant is created.

Iodine

The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants for laboratory use are the iodophors, with Wescodyne being perhaps the most widely used. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water (25 ppm available iodine) to 3 oz. in 5 gal. of water (75 ppm available iodine). The small amount of free iodine available in this range can rapidly be taken up by extraneous protein that may be present. Clean surfaces or clear water can be effectively treated with 75-ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For iodophors such as Wescodyne, it is critical that the manufacturer's written instructions are followed. Higher concentrations of iodophores are actually less effective, as the iodine is bound to itself or the carrier molecule. For washing the hands or for use as a sporicide, it is recommended that Wescodyne be diluted 1 to 10 in 50% ethyl alcohol (a reasonably good decontaminant itself.) This will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

10.3.2 Selecting Chemical Disinfectants

No single chemical disinfectant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical disinfectants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

- ◆ What is the target organism(s)?
- ◆ What disinfectants, in what form, are known to, or can be expected to, inactivate the target organism(s)?
- ◆ What degree of inactivation is required?
- ◆ In what medium is the organism suspended (i.e. simple or complex, on solid or porous surface, and/or airborne)?
- ◆ What is the highest concentration of organisms anticipated to be encountered?
- ◆ Can the disinfectant, either as a liquid, vapor, or gas, be expected to contact the organism and can effective duration of contact be maintained?
- ◆ What restrictions apply with respect to compatibility of materials?

- ◆ What is the stability of the disinfectant in use concentrations, and does the anticipated use situation require immediate availability of the disinfectant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the laboratory is the organism(s) under investigation. Laboratory preparations or cultures usually have titers in excess of those normally observed in nature. Inactivation of these materials presents other problems since agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active moieties of chemical disinfectants. Such interference with the desired action of disinfectants may require higher concentrations and longer contact times than those shown to be effective in the test tube. Similarly, a major portion of the contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information with which to predict the probable virulence of these more resistant cells. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting disinfectants and procedures for their use.

Organisms exhibit a range of resistance to chemical disinfectants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid-containing viruses are relatively susceptible to chemical disinfection. The non-lipid-containing viruses and bacteria with a waxy coating, such as tubercle bacillus, occupy a mid-range of resistance. Spore forms and unconventional (slow) viruses are the most resistant.

A disinfectant selected on the basis of its effectiveness against organisms on any range of the resistance scale will be effective against organisms lower on the scale. Therefore, if disinfectants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other organism generated by laboratory operations, even in higher concentrations, would also be inactivated.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in the table on the following pages. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstruums. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. Individual investigators should conclusively determine the efficacy of any of the disinfectants. It is readily evident that each of the disinfectants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable “drug store” of disinfectants.

10.3.3 Characteristics of Some Liquid Disinfectants Table

Liquid Disinfectant Category	Use Dilution	Inactivates				Important Characteristics											Applications				
		Vegetative Bacteria	Lipoviruses	Non-lipid Viruses	Bacterial Spores	Effective shelf life > 1 week ^c	Corrosive	Flammable	Explosion Potential	Residue	Inactivated by organic matter	Compatible with optics ^d	Skin irritant	Eye irritant	Respiratory irritant	Toxic ^e	Work Surfaces	Dirty Glassware	Portable Equip. Surface Decon.	Fixed Equip. Surface Decon.	Liquids for Discard
Quat. Ammon. Cpd	0.1-2.0%	+	+			+					+	+	+	+		+	+	+	+		
Phenolic Cpd	1.0-5.0%	+	+	b		+	+						+	+		+	+	+	+		
Chlorine Cpd	500 ppm ^a	+	+	+	+		+						+	+	+	+	+	+	+	+	
Iodophor	25-1600 ppm ^a	+	+	+	+	+	+						+	+		+	+	+	+		
Alcohol, Ethyl	70-85%	+	+	b		+		+								+	+	+	+		
Alcohol, Isopropyl	70-85%	+	+	b		+		+								+	+	+	+		
Formaldehyde	0.2-8.0%	+	+	+	+	+							+	+		+	+	+	+		
Glutaraldehyde	2%	+	+	+	+	+							+	+		+	+	+	+		

^a – Available halogen

^b – Variable results dependent on virus

^c – Protected from light and air

^d – Usually compatible, but consider interferences from residues and effects on associated materials such as mounting adhesives

^e - By skin or mouth or both – refer to manufacturer’s literature

Adapted from the *Laboratory Safety Monograph a supplement to the NIH Guidelines for Recombinant DNA Research*

Please note that a contact time of ten minutes is generally used in efficacy testing of disinfectants.

11 Spill Response

This guide outlines the basic procedures for dealing with some of the biological spills that you may encounter in your research laboratory. All lab personnel should refer to the relevant spill response procedures before initiating their experiments.

11.1 Composition of a Basic Spill Kit

Microbiological and biomedical research laboratories should prepare and maintain a biological spill kit. A spill kit is an essential safety item for labs working with microbiological agents classified at Biosafety Level 2 or higher and for groups working with large volumes (≥ 1 liter) of Biosafety Level 1 material. A basic spill kit should include:

- ◆ Concentrated household bleach
- ◆ A spray bottle for making 10% bleach solutions
- ◆ Forceps, autoclavable broom and dust pan, or other mechanical device for handling sharps
- ◆ Paper towels or other suitable absorbent
- ◆ Biohazard autoclave bags for the collection of contaminated spill clean-up items
- ◆ Utility gloves and medical examination gloves
- ◆ Face protection (eye wear and mask, or full face shield)

Additional personal protective equipment, such as Tyvek jump suits and powered air-purifying respirators (PAPR's), may be required for response to spills in Biosafety Level 3 laboratories.

Representatives from the OEHS Occupational Health and Safety section are available if you have any questions regarding biological spill response procedures or decontamination (785 - 3550). All spills in a BL3 laboratory shall be reported to OEHS immediately.

11.2 Biosafety Level 1 (BL1) Spill

- ◆ Notify others in the area, to prevent contamination of additional personnel and environment.
- ◆ Remove any contaminated clothing and wash exposed skin with disinfectant.

11.2.1 Clean-up of BL1 Spill

- ◆ Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill allowing it to mix with spilled material. Allow suitable contact time.
- ◆ Pick up any pieces of broken glass with forceps and place in a sharps container.
- ◆ Discard all disposable materials used to clean up the spill into a biohazard autoclave bag.
- ◆ Wash hands with soap and hand-washing disinfectant.

11.3 Biosafety Level 2 (BL2) Spill

- ◆ Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with a warning sign.
- ◆ Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag.
- ◆ Wash all exposed skin with soap and water.
- ◆ Inform Supervisor, and, if assistance is needed, consult the Biosafety Office (785-3550).

11.3.1 Clean-up of BL2 Spill

- ◆ Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, paper towels, biohazard bags, and forceps).
- ◆ Put on protective clothing (lab coat, face protection, utility gloves, and booties if necessary). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask.
- ◆ Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least a 20 minute contact time.
- ◆ Pick up any sharp objects with forceps and discard in a sharps container. Soak up the disinfectant and spill using mechanical means, such as an autoclavable broom and dustpan, since there may be sharps under the paper towels, and place the materials into a sharps container. Smaller pieces of glass may be collected with cotton or paper towels held with forceps. If no sharps were involved in the spill discard the materials into an autoclave bag.
- ◆ Wipe surrounding areas (where the spill may have splashed) with disinfectant.
- ◆ Soak up the disinfectant and spill, and place the materials into a biohazard bag.
- ◆ Spray the area with 10% household bleach solution and allow to air-dry (or wipe down with disinfectant-soaked towels after a 10-minute contact time). Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.
- ◆ Wash hands and exposed skin areas with disinfectant or antiseptic soap and water.

11.3.2 Blood Spills

For blood or other material with a high organic content and low concentration of infectious microorganisms:

- ◆ Wear gloves, eye protection, and a lab coat.
- ◆ Absorb blood with paper towels and place in a biohazard bag. Collect any sharp objects with forceps or other mechanical device and place in a sharps container.
- ◆ Using a detergent solution, clean the spill site of all visible blood.
- ◆ Spray the spill site with 10% household bleach and allow to air-dry for 15 minutes.
- ◆ After the 15 minute contact time, wipe the area down with disinfectant-soaked paper towels.
- ◆ Discard all disposable materials used to decontaminate the spill and any contaminated personal protective equipment into a biohazard bag.
- ◆ Wash your hands.

11.4 Spill of a Biohazardous Radioactive Material

A biohazardous spill involving radioactive material requires emergency procedures that are different from the procedures used for either material alone. Use procedures that protect you from the radiochemical while you disinfect the biological material.

Before any clean up, consider the type of radionuclide, characteristics of the microorganism, and the volume of the spill. Contact the OEHS Radiation Safety Office (785-3550) for isotope clean-up procedures.

- ◆ Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post a warning sign.
- ◆ Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag labeled with a radioactive materials label or a radioactive waste container labeled with a biohazard label.
- ◆ Wash all exposed skin with disinfectant, following it with a three-minute water rinse.

- ◆ Inform supervisor and Radiation Safety Office of spill, and monitor all exposed personnel for radiation. If assistance is needed in handling the microorganism contact the Biosafety Office at 785-3550.

11.4.1 Clean-Up of a Biohazardous Radioactive Material

- ◆ Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, autoclavable containers, forceps, towel, and sponges), and confirm with the Radiation Safety Office that it is safe to enter the lab.
- ◆ Put on protective clothing (gown, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA-filtered respirator instead of a surgical mask.
- ◆ Cover the area with disinfectant-soaked towels, and carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least 20 minutes contact time.

Do Not use bleach solutions on iodinated material, radioactive gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.

- ◆ Handle any sharp objects with forceps. Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- ◆ Soak up the disinfectant and spill, and place the biologically decontaminated waste, along with all contaminated protective clothing, into an approved radiation container and label it according to Radiation Safety Guidelines. Contaminated protective clothing must also be biologically decontaminated prior to disposal as radioactive waste.

Do Not autoclave the waste unless the Radiation Safety Officer approves this action. If waste can not be autoclaved, add additional disinfectant to ensure biological decontamination of all the materials.

- ◆ Wash hands and exposed skin areas with disinfectant; monitor personnel and spill area for residual radioactive contamination.
- ◆ If skin contamination is found, repeat decontamination procedures under the direction of the Radiation Safety Officer.
- ◆ If the spill area has residual activity, determine if it is fixed or removable and handle accordingly.
- ◆ Discarding items contaminated with radioactive materials:
 - Place the contaminated item(s) on absorbent paper.
 - Spray disinfectant (10% household bleach) on the contaminated areas and allow 20 minute contact time.
 - Wrap the item(s) inside the paper and dispose of as radioactive waste.

12 Select Agents

Select Agents are materials that have been identified by the U.S. Government as agents that have potential use in biological terrorism or warfare. The Department of Health and Human Services (DHHS), through the U.S. Centers for Disease Control and Prevention (CDC), and the Animal Plant Health Inspection Service (APHIS), through the United States Department of Agriculture (USDA) regulate Select Agents in the United States and its territories. Each agency has developed and will maintain a list of Select Agents, including human, animal and plant pathogens, high-risk toxins of biological origin, and prions. The current list of Select Agents is provided below and can be accessed at the CDC and USDA web sites (<http://www.cdc.gov/od/sap/faq.htm> and <http://www.aphis.usda.gov/vs/ncie/bta.html>).

12.1 List of Select Agents

The list of select agents below is current as of March 2004. The most current list can be found at <http://www.cdc.gov/od/sap/docs/salist.pdf>.

HHS NON-OVERLAP SELECT AGENTS AND TOXINS

- Crimean-Congo haemorrhagic fever virus
- Coccidioides posadasii*
- Ebola viruses
- Cercopithecine herpesvirus 1 (Herpes B virus)
- Lassa fever virus
- Marburg virus
- Monkeypox virus
- Rickettsia prowazekii*
- Rickettsia rickettsii*
- South American haemorrhagic fever viruses
 - o Junin
 - o Machupo
 - o Sabia
 - o Flexal
 - o Guanarito
- Tick-borne encephalitis complex (flavi) viruses
 - o Central European tick-borne encephalitis
 - o Far Eastern tick-borne encephalitis
 - o Russian spring and summer encephalitis
 - o Kyasanur forest disease
 - o Omsk hemorrhagic fever
- Variola major virus (Smallpox virus)
- Variola minor virus (Alastrim)
- Yersinia pestis*
- Abrin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Shiga-like ribosome inactivating proteins
- Tetrodotoxin

- Bacillus anthracis*
- Brucella abortus*
- Brucella melitensis*
- Brucella suis* *Burkholderia mallei* (formerly *Pseudomonas mallet*)
- Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)
- Botulinum neurotoxin producing species of *Clostridium*
- Coccidioides immitis*
- Coxiella burnetii*
- Eastern equine encephalitis virus Hendra virus
- Francisella tularensis*
- Nipah Virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus
- Botulinum neurotoxin
- Clostridium perfringens* epsilon toxin
- Shigatoxin
- Staphylococcal enterotoxin
- T-2 toxin

Continued on next page

HIGH CONSEQUENCE LIVESTOCK PATHOGENS AND TOXINS/ SELECT AGENTS (OVERLAP AGENTS)

USDA HIGH CONSEQUENCE LIVESTOCK PATHOGENS AND TOXINS (NON-OVERLAP AGENTS AND TOXINS)

- Akabane virus
- African swine fever virus
- African horse sickness virus
- Avian influenza virus (highly pathogenic)
- Blue tongue virus (Exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- Cowdria ruminantium* (Heartwater)
- Foot and mouth disease virus
- Goat pox virus
- Lumpy skin disease virus
- Japanese encephalitis virus
- Malignant catarrhal fever virus (Exotic)
- Menangle virus
- Mycoplasma capricolum* M.F38/*M. mycoides capri*
- Mycoplasma mycoides mycoides*

- Newcastle disease virus (VVND)
- Peste Des Petits Ruminants virus
- Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus
- Vesicular stomatitis virus (Exotic)

LISTED PLANT PATHOGENS

- Liberobacter africanus*
- Liberobacter asiaticus*
- Peronosclerospora philippinensis*
- Phakopsora pachyrhizi*
- Plum Pox Potyvirus
- Ralstonia solanacearum* race 3, biovar 2
- Schlerophthora rayssiae* var *zeae*
- Synchytrium endobioticum*
- Xanthomonas oryzae*
- Xylella fastidiosa* (citrus variegated chlorosis strain)

12.2 Toxin Amounts Permissible Per Principal Investigator

Regulated toxins of biological origin (see table below) can be ordered, used, or maintained in the laboratory provided the total quantity per PI, for all areas under the PI's control, does not exceed the limits posted in the table below for each toxin. Toxins ordered used, or in the possession of a Yale Principal Investigator cannot exceed the current published maximum allowable exempt quantities (see below) unless both Yale University and the Principal Investigator are registered with the applicable governmental institution.

If the quantity of a toxin under one PI's control exceeds the "OEHS Watch List Quantity" please notify OEHS Biosafety, or the Safety Advisor for the area, to registration as a potential Select Agent Toxin user. OEHS will then inspect your toxin inventory more frequently to check against the maximum allowable exempt quantity.

The list of select agents below is current as of March 2004. The most current list of "Maximum Allowable Quantities" can be found at <http://www.cdc.gov/od/sap/toxinamt.htm>. For current OEHS Watch List Quantities please contact the Biosafety Office at 785-3550 or the area Safety Advisor.

Toxin Amounts Permissible per Principal Investigator

HHS Toxins	Maximum Allowable Exempt Quantity	OEHS Watch List Quantity	Agency(ies)
Abrin	100 mg	50 mg	HHS
Botulinum neurotoxins	0.5 mg	0.25 mg	HHS/USDA
Clostridium perfringens epsilon toxin	100 mg	50 mg	HHS/USDA
Conotoxin	100 mg	50 mg	HHS
Diacetoxyscirpenol (DAS)	1000 mg	500 mg	HHS
Ricin	100 mg	50 mg	HHS
Saxitoxin	100 mg	50 mg	HHS
Shiga-like ribosome inactivating proteins	100 mg	50 mg	HHS
Shigatoxin	100 mg	50 mg	HHS/USDA
Staphylococcal enterotoxins	5.0 mg	2.5 mg	HHS/USDA
Tetrodotoxin	100 mg	50 mg	HHS
T-2 toxin	1000 mg	500 mg	HHS/USDA

12.3 Possession, Use, or Transfer of Select Agents

In order to possess, use, send or receive Select Agents, an institution and each individual who will have access to the Select Agent(s) must first satisfy the following requirements. Each requirement must be approved prior to possession, use or transfer.

Register with the applicable U.S. Governing bodies (CDC, APHIS, and/or USDA) through the Yale Office of Environmental Health & Safety (OEHS).

- ◆ Register with the State of Connecticut Department of Public Health through Yale OEHS. This is required for those Select Agents that are human pathogens.
- ◆ Official authorization granted for each individual requesting access to Select Agents provided by the U.S. Federal Bureau of Investigation, the applicable U.S. Governing body, and Yale University.

Please note that violations of Select Agent rules and regulations can lead to severe criminal or civil penalties. Imprisonment and fines up to 250,000.00 may be levied against individuals who are found in violation of these laws.

12.4 Registration of Possession, Use or Transfer of Select Agents.

All activity involving Select Agents must be registered with the Yale Office of Environmental Health & Safety prior to initiation. Please contact the Biosafety Office with Yale OEHS or contact your OEHS Safety Advisor at 785-3550 to initiate a registration for your proposed Select Agent activity. The following bullets summarize the Select Agent registration and compliance pathway at Yale University.

- ◆ Notify Yale OEHS of your intent to possess, use or transfer Select Agents.
- ◆ Complete an update of your Yale OEHS FORM 01 Registration with the new agents. (If the Select Agent is a human pathogen, Yale will help you also register with the State of CT Dept. of Public Health).
- ◆ Complete Yale Select Agent Patriot Act Declaration Form
- ◆ Complete FBI Form FD961 and file with Yale OEHS for registration with applicable federal entity
- ◆ Complete 2 sets of FBI fingerprint cards for initiation of background investigation check
- ◆ Complete all Yale OEHS applicable training programs (Biosafety, Bloodborne Pathogens, Laboratory Safety, Biosafety Level 3, Select Agent Biosecurity, and Shipping/Transport of Hazardous Biological Agents).

- ◆ Complete a Yale OEHS Request to Use Infectious Agents Form(s) and Researcher Experience Form.
- ◆ Complete the Yale OEHS researcher experience requirements (for BL2+ or BL3 agents)
- ◆ Satisfactory completion of OEHS laboratory inspection of proposed work practices, safety equipment, and facility for Yale, CDC/NIH, and Select Agent regulatory requirements. (Safety and Security)
- ◆ Receive final approval and authorization from Yale OEHS, FBI, and the applicable governing body that you and each individual requesting access to Select Agents, the proposed storage location for Select Agents, and the Select Agent research areas have been cleared. (This is provided in the form of an approval letter from the Yale Biological Safety Committee).

Your laboratory will be subject to Yale and federal inspection or audit prior to initiation of work and at any time during your possession of Select Agents.

12.5 Discovery of Select Agents or Unknown Samples

Please notify OEHS immediately if:

- ◆ You identify any Select Agent pathogen or toxin listed on the current federal list that was not previously registered by your lab
- ◆ You discover a toxin not previously reported by your laboratory in excess of either the Yale Watch List or the federal maximum allowable quantities listed above.
- ◆ You discover any unknown materials in your laboratory for assistance with identification.

These discoveries must be reported to the applicable governmental institution.

12.6 Intrafacility Transfer of Select Agents

Select agent pathogens and toxins may not be transferred outside of, to, or within Yale University unless OEHS and federal approval has been granted. An intrafacility transfer is defined as the transfer of a Select Agent from one OEHS and federally registered Select Agent lab to a similarly registered laboratory. Select Agents may not be transferred to a laboratory that is not registered with OEHS and the applicable governmental institution. Once approved, intrafacility transfers will be overseen by OEHS. Please contact the OEHS Biosafety Office for additional information.

12.7 Destruction of Select Agents or Unknown Samples

Select Agent pathogens or toxins may not be destroyed until Yale OEHS and the applicable government institution has provided approval for the destruction. Once approval has been granted for the destruction of Select Agents, Yale OEHS will officially assume possession of the material and record its destruction. The governing institution will alert Yale if witnesses are required.

If you have any questions regarding the Yale of Federal Select Agent process, please don't hesitate to contact the OEHS Biosafety Office or your OEHS Safety Advisor at 785-3550. In addition, some Select Agents web sites are located below.

Additional Information on the Select Agent Program may be found at the following web sites:

Yale OEHS: <http://www.yale.edu/oehs/>

Centers for Disease Control and Prevention: <http://www.cdc.gov/od/sap/>

Notice of Exclusion for attenuated strains SAs: <http://www.cdc.gov/od/sap/exclusion.htm>

Animal Plant Health Inspection Service: <http://www.aphis.usda.gov/vs/ncie/bta.html>

United States Department of Agriculture: <http://www.usda.gov/>

FBI Form FD961: <http://www.fbi.gov/terrorinfo/fd-961.pdf>

13 Shipping

13.1 Packaging and Shipping of Biomedical Material

Anyone packaging, handling, shipping or transporting hazardous materials must receive training in the general requirements of handling hazardous materials as well as function specific training for the specific task(s) performed. Training is required before performing any tasks associated with shipping hazardous materials and periodically thereafter. In the U.S., this training must be documented for new employees who package, ship or transport hazardous materials within the first 90 days of hire. The level of training varies for the material being shipped and the complexity of the shipment. For example, individuals shipping known human pathogens require more training than individuals who only ship standard clinical specimens that are not known to contain a human pathogen. The United States Department of Transportation (DOT) requires retraining every three years, and the International Air Transportation Association (IATA) requires retraining every two years. Yale University is requiring retraining every two years for anyone shipping biological agents since air transport is routinely involved.

Hazardous materials training must include information on general awareness to familiarize hazardous materials employees (those who package, handle, ship or transport hazardous materials) with the regulations. It must also include function-specific training, safety, and security awareness. Those who will operate a motor vehicle for the transport of hazardous materials also require driver training.

Training information may be found at the following web site: <http://www.yale.edu/oehs/bioreqVII.htm>

Before shipping material from Yale you must complete a "HAZMAT Shipment Request" form and fax back to OEHS at 785-7588. The form may be found at the following web site:

<http://www.yale.edu/oehs/Documents/lab/LABhazmatchecklist.pdf>,

13.1.1 Packaging of diagnostic specimens and biological products (42 CFR Part 72.2)

Such material must be "packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation." This should be interpreted to mean that the contents should not leak to the outside of the shipping container, even if there should be leakage of the primary container(s) during transit, unless the package is severely damaged, e.g., like being run over by a transport vehicle. These packages should, on the other hand, withstand rough handling and passage through cancellation machines, sorters, conveyors, etc.

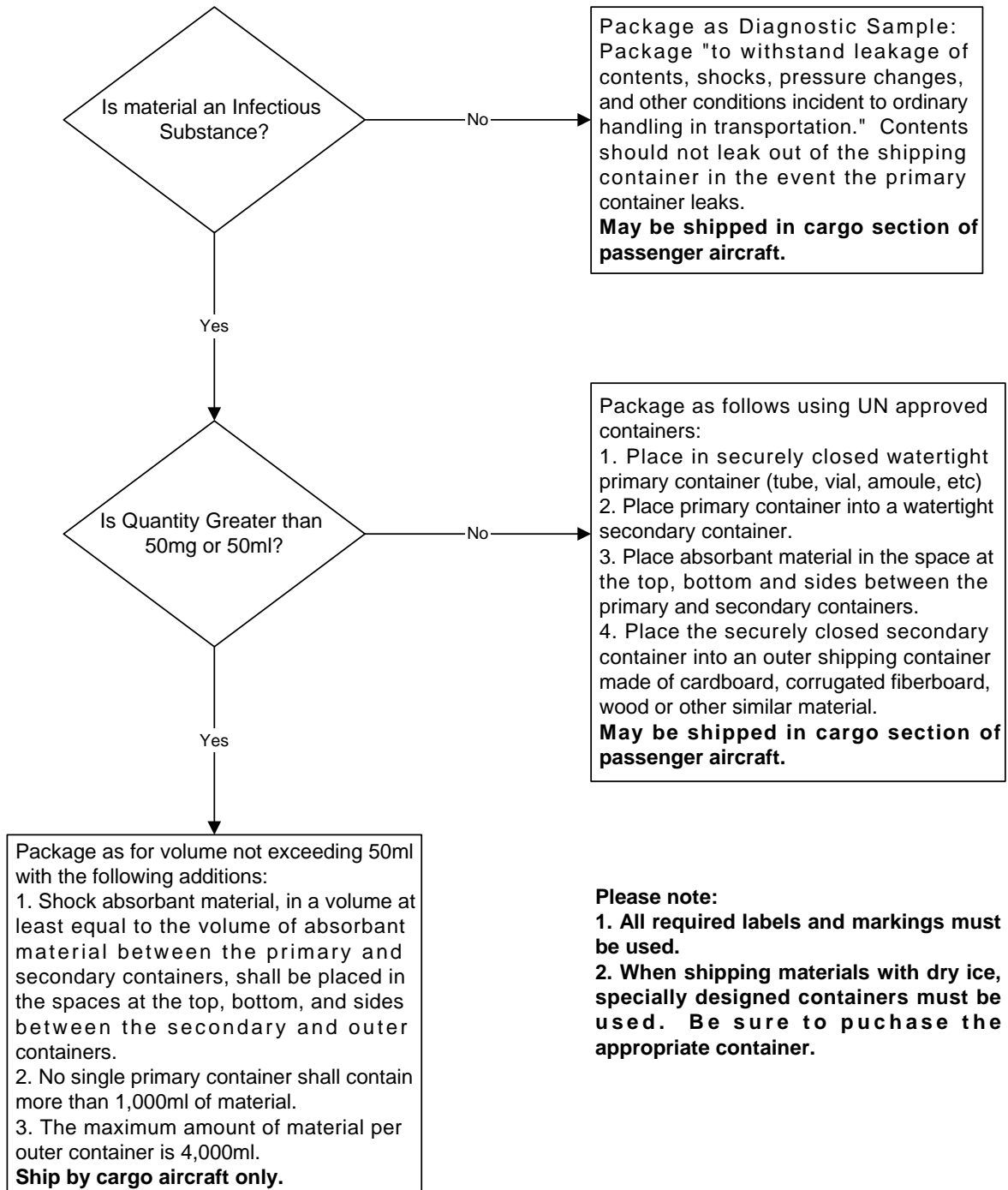
13.1.2 Packaging of materials containing etiologic agents (42 CFR Part 72.3)

A. There are two sets of instructions for this material, depending upon the volume shipped.

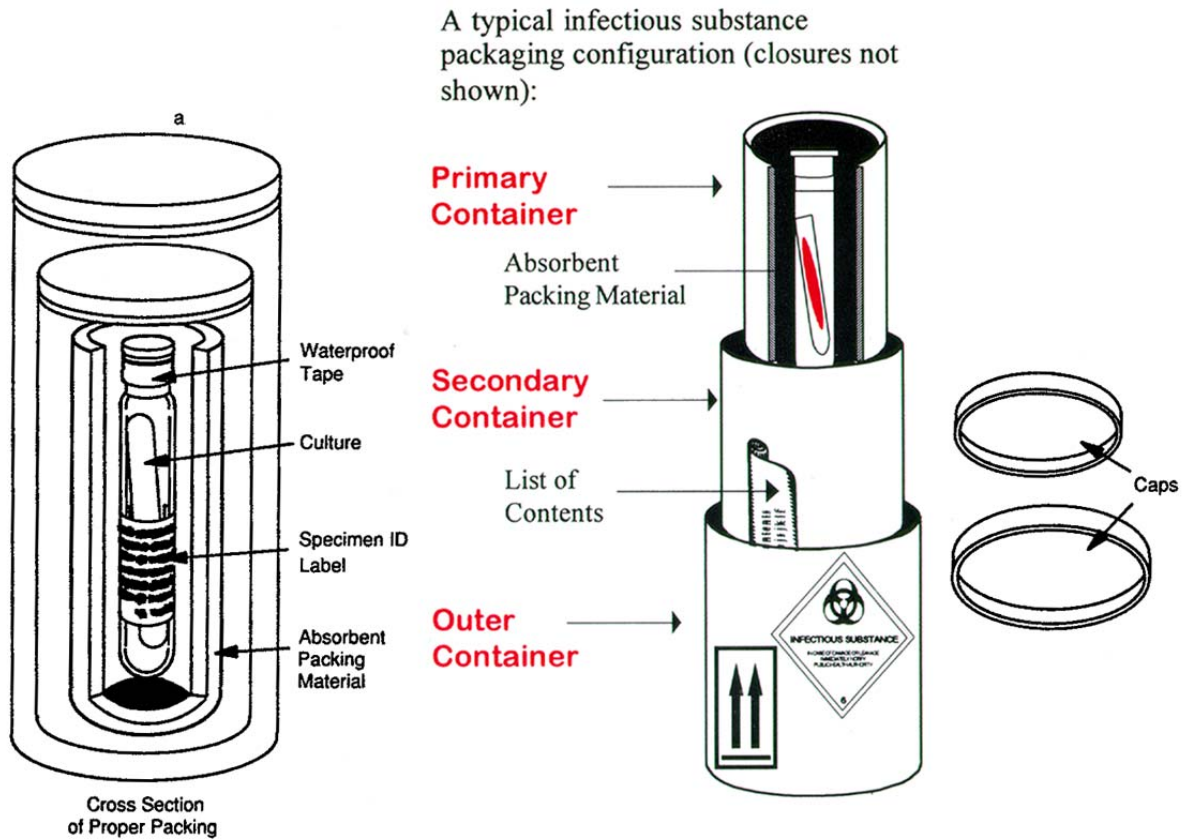
1. Volume not exceeding 50 ml.:
 - a) The material to be shipped shall be placed in a securely closed, watertight tube, vial, ampoule or the like that is referred to as primary container.
 - b) The primary container is then placed in a durable watertight container referred to as the secondary container.
 - c) Several primary containers can be placed in a single secondary container, so long as total contents of the primary containers does not exceed 50 ml.
 - d) Absorbent material must be placed in the space at top, bottom, and sides between the primary and secondary containers. There must be enough absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage and should not be nonparticulate, i.e., not sawdust, vermiculite, etc.

- e) Each set of primary and secondary containers is then placed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength. This means that most, if not all, bags, envelopes, and the like are not acceptable outer shipping containers.
2. Volume greater than 50 ml.:
- Packaging of these larger volumes of material must comply with all of the foregoing requirements, but in addition:
- a) Shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary container and outer shipping container.
 - b) Single primary containers shall not contain more than 1,000 ml of material, however two or more primary containers, whose volumes do not exceed 1,000 ml, may be placed in a single secondary container.
 - c) The maximum amount of etiological agent that may be enclosed within a single outer shipping container may not exceed 4,000 ml.
- B. If dry ice is used, it must be placed between the secondary container(s) and the outer shipping container and the shock absorbent material placed so that the secondary container(s) do not become loose within the outer shipping container as the dry ice sublimates.
- C. A special label, illustrated on page 3, must be placed on the outer shipping container. This label identifies the package as containing etiologic agents and directs anyone observing damage to the package or leakage of contents to call CDC.
- D. The regulation also contains a list of etiological agents that require special handling in addition to that stated above. These are, by and large, Class 3 and Class 4 agents. The requirement is that they be shipped by "registered mail or an equivalent system which requires or provides for sending notification of receipt to the sender immediately upon delivery." When this notice of receipt is not received "within 5 days following anticipated delivery" the sender must notify CDC.

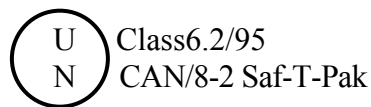
13.1.3 Packaging Flowchart



13.1.4 Infectious Substance Packaging Diagram



Packaging for infectious substances must be United Nations (UN) approved. UN approved packaging bears the UN mark, the manufacturers code, packaging specifications and country of manufacture. An example is below:



13.2 Export of Infectious Materials

The export of infectious material may require a license from the Department of Commerce. OEHS will review each export of biological, chemical or radioactive materials to determine if an export permit is required. If a permit is required OEHS will assist with the process. Before exporting any biological, chemical, or radioactive material please complete a “HAZMAT Shipment Request” form and fax back to OEHS at 785-7588. The form may be found at the following web site:

<http://www.yale.edu/oehs/Documents/lab/LABhazmatchecklist.pdf>

13.3 Importation Permits for Etiologic Agents

Certain materials require import permits prior to entering the United States. Below is a table outlining permit requirement. Most agencies have indicated that if there is doubt whether a permit is required it is best to submit the permit application and the agency will decide. If you have any questions please call the Biosafety Office at 785-3550 or the appropriate agency.

13.3.1 Table of Required Permits

Agency	Material to be Imported
<p>CDC Call (404) 639-3883 for further information</p>	<p>Etiologic agents - infectious agent known to cause disease in man. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts and molds. In some instances, agents which are suspected of causing human disease also require a permit.</p> <p>Biological materials - Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected of being infected with disease transmissible to man.</p> <p>Animals - Any animal known or suspected of being infected with any disease transmissible to man. Importation of turtles of less than 4 inches in shell length and all non-human primates require an importation permit issued by the Division of Quarantine. Telephone (404) 639-1437 for further information.</p> <p>Insects - Any living insect, or other living arthropod, known or suspected of being infected with any disease transmissible to man. Also, if alive, any fleas, flies, lice, mites, mosquitoes, or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.</p> <p>Snails - Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either Centers for Disease Control and Prevention or the Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.</p> <p>Bats - All live bats. Bats may also require a permit from the U.S. Department of Interior, Fish and Wildlife Services.</p>
<p>USDA Call (410) 436-8226 or visit the USDA web site at http://www.aphis.usda.gov for further information</p>	<p>Materials derived from animals or exposed to animal-source materials. Materials which require a permit include animal tissues, blood, cells or cell lines of livestock or poultry origin, RNA/DNA extracts, hormones, enzymes, monoclonal antibodies for IN VIVO use in non-human species, certain polyclonal antibodies, antisera, bulk shipments of test kit reagents, and microorganisms including bacteria, viruses, protozoa, and fungi. Exceptions to this requirement are human and non-human primate tissues, serum, and blood.</p> <p>Dairy products (except butter and cheese), and meat products (e.g., meat pies, prepared foods) from countries with livestock diseases exotic to the U.S.</p> <p>Foreign plant pests injurious to plants grown in the United States.</p> <p>Designated noxious weeds, which are of foreign origin and new to or not widely prevalent in the United States.</p> <p>Insects, Mites, and Nematodes Introduced for Biological Control of Weeds in the United States.</p> <p>Biological control organisms imported, shipped, and released in the United States.</p> <p>Insects and Mites Commonly Included in Shipments as Host Material for Biological Control Agents.</p> <p>Domestic Plant Pests Regulated by Federal or State Quarantines.</p> <p>Low-Risk Organisms, including Arthropods and Pathogens.</p> <p>Nonregulated domestic plant pests shipped into an area in the United States where the pests do not occur.</p>
<p>USDI (Department of Interior) Call (800) 358-2104 for further information</p>	<p>Certain live animals and all live bats</p>

Appendix A Form 01: Registration for the Use of Biological

Materials

Please return to: Office of Environmental Health and Safety
Occupational Health and Safety Section
135 College St.
Tel. 737-2121 / Fax 785-7588

OFFICE USE
BL: _____
BIOREG#: _____

Please print or type:

Principal Investigator: _____ ss#: _____

Office Bldg./Rm. number: _____ Tel #: _____ (office) _____ (lab)

Lab Director: _____ ss#: _____

Lab Supervisor (if other than PI): _____ Tel #: _____ (office) _____ (lab)

List all laboratory rooms used (Bldg./Rm.): _____

Department: _____

Type of laboratory (Check all that apply) Teaching [] Research [] Clinical [] Environmental Analysis []

Are Biological agents used? Yes [] No [] Are Plants used? Yes [] No []

Does your laboratory perform any Recombinant DNA experiments? Yes [] No []

If yes, have you registered your Recombinant DNA experiments? Yes [] No []

If yes, provide your Yale Registration #: _____

If no, are your Recombinant DNA experiments exempt from NIH guidelines? Yes [] No [] (see checklist on reverse side)

Describe specific host(s), vector(s), DNA(s) and what proteins will be produced?

Biological/Biohazardous waste disposed through Yale University Environmental Services [] YNHH []

(Complete the following if Yale University Environmental Services checked)

1. Do you have a copy of Yale Waste Watcher's Guide? [] yes [] no

2. How do you decontaminate biological waste? _____

3. Location of autoclave available for your use _____

4. In addition to immediately contacting Occupational Health & Safety Section, what plans have been made by the Principal Investigator for decontamination in case of a biological spill accident? _____

5. I am aware of the CDC-NIH Biosafety containment levels and precautions applicable to the work described here. I understand that I am responsible for the safety of others in my laboratory, and those who handle the waste generated by my laboratory.

P.I. Signature _____ Date _____

A FORM 01 should be completed annually or whenever information on your current registration changes. A blank FORM 01 may be obtained from the Occupational Health and Safety Section, the Occupational Health and Safety web site (<http://www.yale.edu/oehs/pdfforms.htm>), or your business office.

CHECK LIST TO DETERMINE WHETHER TISSUE CULTURE EXPERIMENTS ARE EXEMPT FROM THE RECOMBINANT DNA GUIDELINES

Many tissue experiments with recombinant DNA molecules are exempt from the NIH Guidelines. If the answer to all 5 of the following questions is no, then the tissue culture experiments are exempt according to Appendix C-I.

- Do any recombinant DNA molecules contain one-half or more of any eukaryotic viral genome?
- Do any experiments involve Class 3, 4 or 5 organisms or nucleic acids from Class 3, 4 or 5 organisms?
- Do any experiments involve introduction of genes coding for molecules toxic for vertebrates?
- Do any experiments involve infectious viruses?
- Do any experiments involve defective viruses in presence of helper viruses?

CHECK LIST TO DETERMINE WHETHER EXPERIMENTS WITH E. COLI K12 AND YEAST ARE EXEMPT FROM THE RECOMBINANT DNA GUIDELINE

Most experiments involving *E. coli* K-12 host vector systems and *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host vector systems are exempt from the NIH Guidelines. If answer to all 3 of the following questions are no, then the experiments are exempt according to Appendix C-II (for *E. coli* K-12) or Appendix C-III (for *Saccharomyces cerevisiae* and *Saccharomyces uvarum*).

- Do any experiments involve Risk Groups 3, 4 or restricted organisms or nucleic acids from Risk Groups 3, 4 or restricted organisms?
- Do any experiments involve introduction of genes coding for molecules toxic for vertebrates?
- Will there be any large-scale experiments (more than 10 liters of culture)?

CHECK LIST TO FIND RELEVANT SECTION OF THE RECOMBINANT DNA GUIDELINES

Section III-D: Experiments that require IBC approval before initiation.

Section III-E: Experiments that require IBC notice simultaneous with initiation.

- Is any human or animal pathogen (defined as a Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents) used as either the host organism or as a vector? Section III-D-1
- Is any DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems? Section III-D-2
- Do recombinant DNA or RNA experiments involve the use of infectious animal or plant viruses in tissue culture systems? Section III-D-3
- Do recombinant DNA or RNA experiments involve the use of defective animal or plant viruses in the presence of helper virus in tissue culture systems? Section III-D-3
- Do recombinant DNA experiments involve whole animals (Section III-D-4) or plants (Section III-D-5)?
- Do experiments involving more than 10 liters of culture? Section III-D-6
- Section III-E: Catchall section for experiments that are not exempt but are not covered in other sections.

Note: Transgenic or knockout rodent experiments that require BL1 containment may be initiated simultaneously with IBC notification. The purchase of transgenic rodents for BL1 experiments is exempt from the NIH Guidelines.

Section III-A: Experiments that require IBC, RAC Review and NIH Director approval before initiation.

Section III-B: Experiments that require NIH/ORDA and IBC approval before initiation.

Section III-C: Experiments that require IRB and IBC approval and NIH/ORDA Registration before initiation.

- III-A-1: Transfer of drug resistance trait to organism that does not acquire it normally.
- III-B-1: Formation of recombinant DNAs containing genes coding for the synthesis of molecules toxic for vertebrates.
- III-C-1: Human gene transfer experiments.

The tables on the following pages refer to the laboratory's use of biological agents. Please complete only those tables that are relevant to the work of the laboratory.

Microorganisms (p.3) []

Biological Safety Cabinets (units housed in following labs):		
Building	Room	Serial Number

- Human Materials** (p.4) []
- Tissue Culture (p.5) []
- Transplantable tumors (p.5) []
- Hybridomas (p.6) []
- Animals/Insects** (p.7) []
- Tissue Culture (p.7) []
- Transplantable Tumors (p.8) []
- Hybridomas (p.8) []
- Biological Toxins** (p.9) []

Please supply social security numbers for all employees working with any type of human materials. A social security number is used to identify occupationally exposed employees in the computer system.

Microorganisms

Containment at Biosafety Level 1 2 3 4 (circle one)

Agent	Host	Route of Inoculation	All Bldg./rms. where used/stored

For each agent, please list all potential handlers below:

Agent: _____
 Handlers: _____

Agent: _____
 Handlers: _____

Agent: _____
 Handlers: _____

Agent: _____
 Handlers: _____

From where are the above agents obtained?

- [] Immediate Colleague
- [] ATCC
- [] Specimen isolated from nature
- [] Other _____

Human Materials

Containment at Biosafety Level 1 2 3 4 (circle one)

Does your laboratory examine any specimens for the purpose of providing information to physicians? Yes [] No []

If yes, what type of organisms do you normally look for? _____

What are the test results used for? research [] diagnostic purposes [] treating patients []

Human Material	Type/Source (applicable)	All Bldg./rms. where used/stored
Blood		
Body Fluids		
Organs		
Tissues		

For each material, please list all potential handlers below:

Material: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Material: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Material: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Material: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

For additional materials, please use separate sheet.

Human Materials continued

Tissue Culture

Containment at Biosafety Level

1 2 3 4 (circle one)

Primary Cell Lines/Continuous Cell Lines (<i>please indicate</i>)	Source	Bldg./Rm. where used/stored

For each cell line, please list all potential handlers below:

Cell line: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Cell line: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Cell line: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Cell line: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

For additional materials, please use separate sheet.

Transplantable tumors

Tumor/Description	Institutional source	Animal or Tissue culture	Containment test date	Bldg./Rm. where used/stored

For each tumor, please list all potential handlers below:

Tumor: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Tumor: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

For additional materials, please use separate sheet.

**Human Materials continued
 Hybridoma**

Containment at Biosafety Level

1 2 3 4 (circle one)

Carrier cell line	<i>In vivo/In vitro</i>	Specify animals used	Bldg./Rm. where used/stored/housed

For each Hybridoma, please list all potential handlers below:

Hybridoma: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Hybridoma: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Hybridoma: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

Hybridoma: _____
 Handlers: 1.) _____
 ss# _____
 2.) _____
 ss# _____
 3.) _____
 ss# _____
 4.) _____
 ss# _____

For additional materials, please use separate sheet.

Animals or Insects Continued

Containment at Biosafety Level

1 2 3 4 (circle one)

Transplantable tumors [] yes [] no

Tumor/Description	Institutional source	Animal or Tissue culture	Containment test date	Bldg./Rms. where used/stored

For each tumor, please list all potential handlers below:

Tumor: _____
 Handlers: _____

Tumor: _____
 Handlers: _____

Tumor: _____
 Handlers: _____

Tumor: _____
 Handlers: _____

For additional materials, please use separate sheet.

Hybridoma

Carrier cell line	<i>In vivo/In vitro</i>	Specify animals used	Bldg./Rms. where used/stored/housed

For each Hybridoma, please list all potential handlers below:

Hybridoma: _____
 Handlers: _____

Hybridoma: _____
 Handlers: _____

Hybridoma: _____
 Handlers: _____

Hybridoma: _____
 Handlers: _____

For additional materials, please use separate sheet.

Biological Toxins

Containment at Biosafety Level

1 2 3 4 (circle one)

Type of Toxin	Experimental Concentration	Supplier	All Bldg./rms. where used/stored
Aflatoxins			
Amanitin			
Bacterial Toxins			
Bee Venoms			
Castorbean			
Insect Toxins			
Lectins			
Mycotoxins			
Snake venoms			
Tetrodotxin			
Other			

For each toxin, please list all potential handlers below and on the following page:

Toxin: _____
 Handlers: _____

Toxin: _____
 Handlers: _____

Toxin: _____
 Handlers: _____

Toxin: _____
 Handlers: _____

For additional toxins, please use separate sheet.

Antidote available [] yes [] no Source _____

Antidote in your possession [] yes [] no

Plan to inactivate toxin _____

Toxin LD50

(Human)

(animal)

Appendix B Classification of Human Etiologic Agents on the Basis of Hazard

This section has been reprinted from the NIH *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, October 1997.

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (see Sections V-C, V-D, V-E, and V-F, Footnotes and References of Sections I through IV). Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the NIH Guidelines.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions), *Escherichia coli*-K12 (see Appendix C-II-A, *Escherichia coli* K-12 Host-Vector Systems, Exceptions), and adeno-associated virus types 1 through 4.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.

Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

- ◆ *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) except those listed in Appendix B-III-A (RG3)
 - ◆ *Campylobacter coli*, *C. fetus*, *C. jejuni*
 - ◆ *Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
 - ◆ *Clostridium botulinum*, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. histolyticum*, *Cl. novyi*, *Cl. septicum*, *Cl. tetani*
 - ◆ *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* O157:H7
 - ◆ *Haemophilus ducreyi*, *H. influenzae*
 - ◆ *Helicobacter pylori*
 - ◆ *Klebsiella* - all species except *K. oxytoca* (RG1)
 - ◆ *Legionella* including *L. pneumophila*
 - ◆ *Leptospira interrogans* - all serotypes
 - ◆ *Listeria*
 - ◆ *Moraxella*
 - ◆ *Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum*, *M. bovis* BCG vaccine strain, *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* which are restricted animal pathogens
 - ◆ *Neisseria gonorrhoeae*, *N. meningitidis*
 - ◆ *Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
 - ◆ *Rhodococcus equi*
 - ◆ *Salmonella* including *S. arizonae*, *S. cholerasuis*, *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*
 - ◆ *Staphylococcus aureus*
 - ◆ *Streptobacillus moniliformis*
 - ◆ *Streptococcus* including *S. pneumoniae*, *S. pyogenes*
 - ◆ *Treponema pallidum*, *T. carateum*
 - ◆ *Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
 - ◆ *Yersinia enterocolitica*
-

Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents

- ◆ *Blastomyces dermatitidis*
- ◆ *Cladosporium bantianum*, *C. (Xylohypha) trichoides*
- ◆ *Cryptococcus neoformans*
- ◆ *Dactylaria galopava* (*Ochroconis gallopavum*)
- ◆ *Epidermophyton*
- ◆ *Exophiala (Wangiella) dermatitidis*
- ◆ *Fonsecaea pedrosoi*
- ◆ *Microsporium*
- ◆ *Paracoccidioides braziliensis*
- ◆ *Penicillium marneffeii*
- ◆ *Sporothrix schenckii*
- ◆ *Trichophyton*

Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents

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

Appendix B-II-D. Risk Group 2 (RG2) - Viruses

- ◆ Adenoviruses, human - all types
 - ◆ Alphaviruses (Togaviruses) - Group A Arboviruses
 - Eastern equine encephalomyelitis virus
 - Venezuelan equine encephalomyelitis vaccine strain TC-83
 - Western equine encephalomyelitis virus
 - ◆ Arenaviruses
 - Lymphocytic choriomeningitis virus (non-neurotropic strains)
 - Tacaribe virus complex
 - Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
 - ◆ Bunyaviruses
 - Bunyamwera virus
 - Rift Valley fever virus vaccine strain MP-12
 - Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
 - ◆ Calciviruses
 - ◆ Coronaviruses
 - ◆ Flaviviruses (Togaviruses) - Group B Arboviruses
 - Dengue virus serotypes 1, 2, 3, and 4
 - Yellow fever virus vaccine strain 17D
 - Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
 - ◆ Hepatitis A, B, C, D, and E viruses
 - ◆ Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, Risk Group 4 (RG4) -Viral Agents)
 - Cytomegalovirus
 - Epstein Barr virus
 - Herpes simplex types 1 and 2
 - Herpes zoster
 - Human herpesvirus types 6 and 7
 - ◆ Orthomyxoviruses
 - Influenza viruses types A, B, and C
 - Other tick-borne orthomyxoviruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
 - ◆ Papovaviruses
 - All human papilloma viruses
 - ◆ Paramyxoviruses
 - Newcastle disease virus
 - Measles virus
 - Mumps virus
 - Parainfluenza viruses types 1, 2, 3, and 4
 - Respiratory syncytial virus
 - ◆ Parvoviruses
 - Human parvovirus (B19)
 - ◆ Picornaviruses
 - Coxsackie viruses types A and B
 - Echoviruses - all types
 - Polioviruses - all types, wild and attenuated
-

- Rhinoviruses - all types
- ◆ Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, Risk Group 3 (RG3) - Viruses and Prions) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, Footnotes and References of Sections I through IV)
- ◆ Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
- ◆ Rhabdoviruses
 - Rabies virus - all strains
 - Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow
- ◆ Togaviruses (see Alphaviruses and Flaviviruses)
 - Rubivirus (rubella)

Appendix B-III. Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

- ◆ Bartonella
- ◆ Brucella including *B. abortus*, *B. canis*, *B. suis*
- ◆ Burkholderia (*Pseudomonas*) *mallei*, *B. pseudomallei*
- ◆ *Coxiella burnetii*
- ◆ *Francisella tularensis*
- ◆ *Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia), *M. tuberculosis*
- ◆ *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*

Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents

- ◆ *Coccidioides immitis* (sporulating cultures; contaminated soil)
- ◆ *Histoplasma capsulatum*, *H. capsulatum* var. *duboisii*

Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents

None

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

- ◆ Alphaviruses (Togaviruses) - Group A Arboviruses
 - Semliki Forest virus
 - St. Louis encephalitis virus
 - Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))
 - Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
 - ◆ Arenaviruses
 - Flexal
 - Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
 - ◆ Bunyaviruses
 - Hantaviruses including Hantaan virus
-

- Rift Valley fever virus
- ◆ Flaviviruses (Togaviruses) - Group B Arboviruses
 - Japanese encephalitis virus
 - Yellow fever virus
 - Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
- ◆ Poxviruses
 - Monkeypox virus
- ◆ Prions
 - Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, Footnotes and References of Sections I through IV, for containment instruction)
- ◆ Retroviruses
 - Human immunodeficiency virus (HIV) types 1 and 2
 - Human T cell lymphotropic virus (HTLV) types 1 and 2
 - Simian immunodeficiency virus (SIV)
- ◆ Rhabdoviruses
 - Vesicular stomatitis virus

Appendix B-IV. Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents

None

Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents

None

Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents

None

Appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents

- ◆ Arenaviruses
 - Guanarito virus
 - Lassa virus
 - Junin virus
 - Machupo virus
 - Sabia
 - ◆ Bunyaviruses (Nairovirus)
 - Crimean-Congo hemorrhagic fever virus
 - ◆ Filoviruses
 - Ebola virus
 - Marburg virus
 - ◆ Flaviviruses (Togaviruses) - Group B Arboviruses
 - Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses
 - ◆ Herpesviruses (alpha)
 - Herpesvirus simiae (Herpes B or Monkey B virus)
-

- ◆ Paramyxoviruses
 - Equine morbillivirus
- ◆ Hemorrhagic fever agents and viruses as yet undefined

Appendix B-V. Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 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 RG2 human agents is recommended.

- ◆ Baculoviruses
- ◆ Herpesviruses
 - Herpesvirus ateles
 - Herpesvirus saimiri
 - Marek's disease virus
 - Murine cytomegalovirus
- ◆ Papovaviruses
 - Bovine papilloma virus
 - Polyoma virus
 - Shope papilloma virus
 - Simian virus 40 (SV40)
- ◆ Retroviruses
 - Avian leukosis virus
 - Avian sarcoma virus
 - Bovine leukemia virus
 - Feline leukemia virus
 - Feline sarcoma virus
 - Gibbon leukemia virus
 - Mason-Pfizer monkey virus
 - Mouse mammary tumor virus
 - Murine leukemia virus
 - Murine sarcoma virus
 - Rat leukemia virus

Appendix B-V-1. Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Appendix C Biosafety Levels

Only work at biosafety levels 1,2 and 3 is allowed at Yale University. There are no biosafety level 4 facilities or biosafety level 4 work allowed at Yale University.

The CDC and NIH have established biosafety guidelines that are found in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). The following is reprinted from that publication. For additional information please contact the Biosafety Office at 785-3550.

Principles of Biosafety

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

LABORATORY PRACTICE AND TECHNIQUE. The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered and that specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

SAFETY EQUIPMENT (PRIMARY BARRIERS). Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A of the CDC/NIH publication. Class I and Class II biological safety cabinets are primary barriers that offer significant levels of protection to laboratory personnel and the environment when used with good microbiological techniques. The Class II biological safety cabinet

also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment. An example of another primary barrier is the safety centrifuge cup; an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this hazard, containment controls such as BSCs or centrifuge cups must be used for handling infectious agents that can be transmitted through the aerosol route of exposure.

Safety equipment also may include items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with biological safety cabinets and other devices that contain the agents, animals, or materials being worked with. In some situations in which it is impractical to work in biological safety cabinets, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

FACILITY DESIGN (SECONDARY BARRIERS). The design of the facility is important in providing a barrier to protect persons working inside and outside of the laboratory within the facility, and to protect persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand-washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules for isolation of the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the Applications Handbook for Heating, Ventilation, and Air-Conditioning (HVAC) published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).

BIOSAFETY LEVELS. Four biosafety levels (BSLs) are described which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity.

BIOSAFETY LEVEL 1 practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, and infectious canine hepatitis virus are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand-washing.

BIOSAFETY LEVEL 2 practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the Bloodborne Pathogen Standard for specific, required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme precaution with contaminated needles or sharp instruments must be emphasized. Even though organisms routinely manipulated at BSL2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate such as splash shields, face protection, gowns, and gloves.

Secondary barriers such as hand-washing and waste decontamination facilities must be available to reduce potential environmental contamination.

BIOSAFETY LEVEL 3 practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

ANIMAL FACILITIES. Four biosafety levels are also described for activities involving infectious disease work with experimental mammals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment.

CLINICAL LABORATORIES. Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory which realistically address the issue of the infective hazard of clinical specimens. Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be done safely at Biosafety Level 2, the recommended level for work with bloodborne pathogens such as hepatitis B virus and HIV. The containment elements described in Biosafety Level 2 are consistent with the Occupational Exposure to Bloodborne Pathogens Standard from the Occupational Safety and Health Administration (OSHA), that requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets should also be used for the initial processing of clinical specimens when the nature of the test requested or other information is suggestive that an agent readily transmissible by infectious aerosols is likely to be present (e.g., *M. tuberculosis*), or when the use of a biological safety cabinet (Class II) is indicated to protect the integrity of the specimen. The segregation of clinical laboratory functions and limiting or restricting access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

Appendix D BL2+ Work Practices

Biosafety Level 2 Plus (BL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BL2 procedures. Generally, BL3 practices are mandated in a space designed for BL2 work. It is preferred that the BL2 laboratory be self-contained; that is, all equipment required for the experiment should be located within the lab. A sign is posted on the door while BL2+ work is in progress, and access is restricted to those involved in the experiment. When work is completed, and equipment has been decontaminated, the sign is removed and the lab returns to standard BL2 or BL1 usage.

BL3 practices require that all work be conducted under physical containment. Therefore, all manipulations with BL2+ material are conducted within a Class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures. The following notes further describe the requirements for work at BL2+.

Personal Protective Equipment (PPE)

- ◆ Dedicate PPE for the experiment. PPE worn for BL2+ work should not be worn in other areas. Remove before leaving the laboratory.
- ◆ Wear a lab coat or solid-front gown, preferably with a knit or grip cuff.
- ◆ Double glove for all work within the biological safety cabinet (BSC). Remove the outer pair before exiting the BSC, and don a new pair each time you reenter the BSC.
- ◆ Ensure that your gloves extend over the sleeve of your lab coat. An opening at the wrist will allow aerosols generated within the BSC to contaminate your wrist and forearm, extending hand-washing to your elbow.
- ◆ Sleeve covers can be worn to ensure coverage of the wrist and will also minimize contamination of the sleeves of your lab coat.
- ◆ Face Protection (mask and eyewear, can also be worn and will protect mucous membranes from exposure in the event a spill outside the BSC during transfer of material to and from the incubator. It will also help to prevent you from touching your eyes, nose and mouth when working within the BSC.
- ◆ Remove PPE before leaving the laboratory. Placing a coat hook within the BL2+ area will facilitate this requirement. Remove your outer gloves first, then your lab coat or gown, followed by the inner gloves. Take your face protection off last. Don't touch your face with gloved hands. Remove gloves and other clothing aseptically, from the inside out, and avoid touching the contaminated outer side of the glove.
- ◆ Decontaminate reusable PPE as soon as feasible after it has been contaminated. Small areas can be spot treated with a suitable disinfectant, such as 1-10% household bleach. Lab coats can also be autoclaved, or sent to a laundry facility equipped to handle biohazardous PPE. Disposable PPE can be placed within a biohazard bag, treated and discarded as biomedical waste.
- ◆ Wash your hands with soap and water after removing PPE and before leaving the laboratory.

Work Practices In the Biological Safety Cabinet (BSC)

- ◆ Perform all work within a BSC. This includes discarding waste within the BSC. Moving your hands in and out of the BSC will disrupt the protective air curtain at the front access opening.
- ◆ Place all items required for the experiment within the BSC before starting work.
- ◆ Wipe items down with disinfectant prior to placement within the BSC.
- ◆ Segregate clean areas from contaminated areas within the BSC (by at least 12-14").
- ◆ Keep the front and rear grilles clear when working within the BSC. Avoid blocking the rear grille. Don't store items on top of the BSC. Remind fellow researchers to minimize traffic and work behind the operator, as this may interfere with cabinet airflow. Depending on the location of the BSC within the room, opening and closing the room door can significantly interfere with BSC airflow.

- ◆ Avoid the use of a flame within the BSC. In addition to presenting a fire hazard, an open flame can disrupt airflow and possibly damage the paper filter located above the work surface. If the use of flame is absolutely necessary, use a burner with a pilot light that provides a flame only when depressed and releases after contact. Never leave an open flame (burner or pilot light) unattended in your BSC.
- ◆ Store tissue culture flasks in the incubator within small secondary trays to help minimize contamination. Trays will also facilitate transfer to and from the BSC.
- ◆ Keep your hands away from your face (face protection helps to minimize the potential for this route of exposure).
- ◆ Avoid the use of glass Pasteur pipettes or needles and syringes. Substitute plastic for glass whenever feasible. Alternatives to glass Pasteur pipettes include: plastic pipettes, plastic transfer pipettes, plastic gel loading pipette tips and pipette tip extendors, aspirators, and flexible plastic aspiration pipettes. Some researchers will either score and break the end off of a 1 ml or 5 ml plastic pipette or remove the wool plug and use for aspirating cultures.
- ◆ If the use of sharps cannot be avoided, maintain a sharps container in the immediate vicinity of use. Discard intact needles and syringes immediately after use. Use a one-handed disposal method (keep a hand behind your back or by your side, don't place on or near the opening of the sharps container). Never recap, bend, break or otherwise manipulate sharps by hand. If you must remove the needle from the syringe, use the small opening on the top of the needlebox for this purpose. Forceps, tweezers, or small pliers may also be utilized.
- ◆ Protect the house vacuum system or pump from contamination by installing a trap and filter system. Use a primary collection flask containing disinfectant, followed by an overflow flask, which leads through a HEPA or hydrophobic filter. Please see the Vacuum System Protection Handout. Vacuum filters are available in the Medical School Stockroom, SHM IE-7.
- ◆ Collect all waste within the BSC. Smaller biohazard waste bags may be utilized along with beakers or shallow trays containing disinfectant for the collection and disinfection of pipettes and other contaminated items. Waste can also be collected within the BSC in the following manner.
 - Horizontal collection: Horizontal trays containing disinfectant allow total immersion of pipettes.
 - Vertical collection: Beakers containing disinfectant can be used if disinfectant is drawn up inside the pipette and allowed to run down the interior wall upon disposal into the beaker.
 - Bags: Bags have the potential for creating aerosols when moved. At BL2+, seal autoclave bags within the cabinet and place within a second bag. Carefully add water to the primary bag before sealing (25 ml for smaller bags, 200 ml for larger bags). The addition of water will help to generate steam within the bag during the autoclave cycle.
- ◆ Wipe items down with disinfectant prior to removal from the BSC.
- ◆ Wipe down BSC with disinfectant after use (work surface, grilles, sides, back and inside front view screen).
- ◆ Decontaminate liquid waste with household bleach diluted 10% against the volume of the waste. Allow at least a 30 minute contact time for full decontamination.
- ◆ Transport waste to autoclave in a leakproof container.

Centrifugation

- ◆ Use sealed rotors or safety buckets as secondary containment for centrifugation.
- ◆ Load and unload the rotor or safety buckets within the BSC.
- ◆ Don't overfill primary containers, limit to $< \frac{3}{4}$ full. Wipe exterior of tube with disinfectant before loading.
- ◆ Seal rotor or bucket and wipe down with disinfectant, remove outer gloves, and transport to the centrifuge.
- ◆ Post a sign on centrifuge that includes the biohazard symbol, name of the agent with Biosafety Level, and your name.

- ◆ Wait 2-5 minutes after the run to allow aerosols to settle in the event of a spill. Transport sealed rotor or safety bucket to cabinet to complete your experiment. Don new pair of outer gloves.
- ◆ Decontaminate the rotor or safety bucket by spraying with 70% ethanol and allowing to air dry. Wipe the throw line within the centrifuge with disinfectant and remove your biohazard sign. In the event of a spill during centrifugation, follow the spill response procedures outlined in the Biosafety Spill Response Guide.
- ◆ Avoid the use of microfuge, which is difficult to contain. If you cannot avoid using a microfuge, use a model that has built in secondary containment (a sealed rotor) along with microfuge tubes equipped with a O-ring seal. You can also operate your microfuge in the rear of your BSC (don't perform any work within the BSC while the microfuge is in operation, and wait 2-5 minutes after the run before opening the microfuge).

Labels

- ◆ Post a biohazard sign at the entry to the BL2+ laboratory.
- ◆ Ensure that any specific entry requirements (vaccination), the name of the agent, the Biosafety Level, and the name of an emergency contact person ARE posted on either the sign or the Laboratory Information Card.
- ◆ Place the BL2 wall notice (not a door sign) inside your laboratory to remind researchers of the core safety practices.
- ◆ Label equipment housing the agent (incubators, freezers) with the universal biohazard symbol and agent name.

Transport of Biohazards on Campus (between labs or buildings):

- ◆ Must have two leakproof containers, including the following:
 - a sealed primary container
 - a sealed secondary container
 - absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
 - a biohazard sticker on the outside of the secondary container with agent name
 - lab address and phone number on the outside of the secondary container
- ◆ Utilize plastic containers whenever feasible. Avoid glass.
- ◆ Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.
- ◆ If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.
- ◆ Decontaminated the outside of the primary container before placing into the secondary container.
- ◆ Decontaminate the secondary container before leaving the laboratory

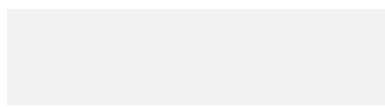
Hand-washing

- ◆ Wash hands whenever PPE is removed and before leaving the laboratory.
- ◆ Wash with soap and warm water for at least 15 seconds. Since the contact time of most soaps is quite extensive for actual decontamination, mechanical friction from scrubbing and water dilution are essential for complete cleaning.
- ◆ No glove is 100% leakproof.
- ◆ Never wet or handwash your gloves with water or disinfectant, as this will encourage wicking and increase permeability of the protective barrier.

Spills and Exposure Incidents

- ◆ All researchers must be familiar with the applicable exposure response procedures before initiating their experiments.
- ◆ Review the attached Biosafety Spill and Incident Response Guide before starting work.

Appendix E Recombinant DNA Registration Form



Complete and send original to:

Office of Environmental Health and Safety
Occupational Health and Safety Section
135 College Street, 1st Floor
New Haven, CT 06510
Phone 785-3550 fax: 785-7588

YALE UNIVERSITY
BIOLOGICAL SAFETY COMMITTEE
REGISTRATION OF RECOMBINANT DNA EXPERIMENTS

Principal Investigator: _____ Anticipated Starting Date: _____

Faculty Rank or Research Appointment: _____

Department: _____ Telephone #: _____ Fax #: _____

Short Title of Proposal: _____

Brief summary stated in non-technical language: _____

I attest that the information in the attached Registration is accurate and complete. I am familiar with and agree to comply with the NIH Recombinant DNA Guidelines and accept the responsibilities listed in Section IV-B-7 as printed on the reverse side of this form, as well as any modifications of these guidelines subsequently issued by the Federal Government or Yale University.

As the Principal Investigator, I agree to accept responsibility for training all laboratory workers involved in the project. All research personnel will be familiar with and understand the potential biohazards and relevant biosafety practices, techniques and emergency procedures.

Written reports will be submitted to the Biological Safety Officer, the Biological Safety Committee, and the Office of Recombinant DNA Activities at NIH (if applicable) concerning: any research related accident, exposure incident or release of rDNA materials to the environment; problems pertaining to the implementation of biological and physical containment procedures; or violations of the NIH Guidelines.

I will not conduct the work described in the attached registration until it has been filed with, and if necessary, approved by the Biological Safety Committee.

Principal Investigator _____ Date _____

Additional Investigator _____ Date _____

Reviewed and Accepted _____ Date _____

I. SPECIFIC INFORMATION:

A. Will experiment be carried out in *E. coli* or other prokaryotic host? Yes _____ No ___

If yes, specify: _____

Host strains: _____

Vectors: _____

Inserted DNA (include names of genes and organisms from which they were cloned): _____

Will whole virus or provirus be cloned? Yes _____ No ___

NIH Guideline Section: _____ Recommended Biosafety Level: _____

B. Will experiment be carried out in eukaryotic cells? Yes _____ No ___

If yes, specify: _____

Host cells: _____

Vectors: _____

Inserted DNA: _____

Helper virus or packaging cells if used: _____

What fraction of an eukaryotic viral genome is contained in the recombinant DNA molecules (including vector

and insert)? Check appropriate range: <1/2 _____ >1/2 but <2/3 _____ >2/3 ___

NIH Guideline Section: _____ Recommended Biosafety Level: _____

C. Will experiment be carried out using whole plants or animals as hosts? Yes _____ No ___

If yes, specify: _____

Plant or animal hosts: _____

Vectors: _____

Inserted DNA: _____

What fraction of an eukaryotic viral genome is contained in the recombinant DNA molecule?

<2/3 _____ >2/3 _____

Will transgenic plants or animals be constructed or used? Yes _____ No ___

NIH Guideline Section: _____ Recommended Biosafety Level: _

II. DESCRIPTION OF EXPERIMENT:

- 1) Include sufficient detail to clarify the scientific basis of the work.
- 2) Note approximate start date for each phase of the work.
- 3) Give references if appropriate.

III. DESCRIBE THE BIOHAZARD POTENTIAL OF THESE EXPERIMENTS:

Are special medical surveillance practices recommended?

IV. LOCATION (Buildings, Room #'s):

V. PERSONNEL (Names, Status and Telephone):

RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR

Section IV-B-7. Principal Investigator (PI). On behalf of the institution, the Principal Investigator is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research.

Section IV-B-7-a. General Responsibilities. As part of this general responsibility, the Principal Investigator shall:

Section IV-B-7-a-(1). Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Section III-A, III-B, and III-C) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;

Section IV-B-7-a-(2). Determine whether experiments are covered by Section III-D and that the appropriate procedures are followed;

Section IV-B-7-a-(3). Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable) within 30 days (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838);

Section IV-B-7-a- (4). Report any new information bearing on the *NIH Guidelines* to the Institutional Biosafety Committee and to NIH/ORDA (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838);

Section IV-B-7-a- (5). Be adequately trained in good microbiological techniques;

Section IV-B-7-a- (6). Adhere to Institutional Biosafety Committee-approved emergency plans for handling accidental spills and personnel contamination; and

Section IV-B-7-a- (7). Comply with shipping requirements for recombinant DNA molecules (see Appendix H for shipping requirements and the *Laboratory Safety Monograph* for technical recommendations).

Section IV-B-7-b. Submissions by the Principal Investigator to the NIH/ORDA. The Principal Investigator shall:

Section IV-B-7-b-(1). Submit information to NIH/ORDA for certification of new host-vector systems;

Section IV-B-7-b-(2). Petition NIH/ORDA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the *NIH Guidelines*;

Section IV-b-7-b-(3). Petition NIH/ORDA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in sections III-A and III-B of the *NIH Guidelines*;

Section IV-B-7-b-(4). Petition NIH/ORDA for determination of containment for experiments requiring case-by-case review; and

Section IV-B-7-b-(5). Petition NIH/ORDA for determination of containment for experiments not covered by the *NIH Guidelines*.

Section IV-B-7-b- (6). Ensure that all aspects of Appendix M, *Points to Consider in the Design and Submission of Protocols for the transfer to Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)*, have been appropriately addressed prior to submission of human gene therapy experiments to NIH/ORDA.

Section IV-B-7-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee. The Principal Investigator shall:

Section IV-B-c- (1). Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;

Section IV-B-c- (2). Select appropriate microbiological practices and laboratory techniques to be used for the research;

Section IV-B-7-c- (3). Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Section III-A, III-B, III-C, or III-D, to the Institutional Biosafety Committee for review and approval or disapproval; and

Section IV-B-7-c- (4). Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

Section IV-B-7-d. Responsibilities of the Principal Investigator Prior to Initiating Research. The Principal Investigator shall:

Section IV-B-7-d- (1). Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

Section IV-B-7-d- (2). Instruct and train laboratory staff in: (I) the practices and techniques required to ensure safety, and (II) the procedures for dealing with accidents; and

Section IV-B-7-d- (3). Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Section IV-B-7-e. Responsibilities of the Principal Investigator during the Conduct of the Research. The Principal Investigator shall:

Section IV-B-7-e- (1). Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

Section IV-B-7-e- (2). Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), the Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable) (reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838);

Section IV-B-7-e- (3). Correct work errors and conditions that may result in the release of recombinant DNA materials; and

Section IV-B-7-e- (4). Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

Section IV-B-7-e- (5). Comply with reporting requirements for human gene transfer experiments conducted in compliance with the *NIH Guidelines* (see Appendix M-VII, *Reporting Requirements – Human Gene Transfer Protocols*).

REGISTRATION OF EXPERIMENTS INVOLVING

RECOMBINANT DNA

YALE BIOLOGICAL SAFETY COMMITTEE

OCTOBER 2000

This outline is intended to serve as an overview of the "Guidelines for Research Involving Recombinant DNA Molecules" (NIH Guidelines.) It is the responsibility of each Yale investigator to make sure that his/her laboratory is in compliance. If your experiments require registration, check the NIH Guidelines for the appropriate biosafety level and relevant section. For additional information, copies of the NIH Guidelines or registration forms, or if you are unsure into which category your experiments fall, please call the Office of Environmental Health and Safety at 785-3550.

Office of Environmental Health and Safety:

Main office; (203) 785-3550

Fax: (203) 785-7588

Website: <http://www.yale.edu/oehs/>

Experiments which must be registered and approved prior to initiation

- 1.) Deliberate transfer of a drug trait to a microorganism not known to acquire it naturally (if it could compromise the use of the drug to control disease agents in humans, animal or agriculture);
- 2.) Human gene transfer experiments;
- 3.) Cloning of DNA encoding molecules lethal to vertebrates at an LD 50 of <100ug/kg body weight;
- 4.) Cloning using human or animal pathogens as host-vector systems;
- 5.) Cloning of DNA from all Risk Group 3, 4 or restricted human or animal pathogens (including HIV and related viruses, and human tumor viruses);
- 6.) Experiments using more than 2/3 of the genome of infectious animal or plant viruses or defective viruses grown in the presence of helper virus;
- 7.) Recombinant DNA experiments involving whole animals or plants;
- 8.) Large scale DNA project (≥ 10 liters of culture combined).

Experiments that require registration simultaneous with initiation:

- 1.) Experiments using as vectors $\leq 2/3$ of the genome of a eukaryotic virus, free of helper virus;
- 2.) Low risk plant rDNA experiments
- 3.) BL1 transgenic or knockout rodent experiments. (Note: the purchase of transgenic rodents for BL1 experiments is exempt from registration);
- 4.) All experiments not specified on this sheet.



Exempt experiments that do not require registration:

- 1.) rDNA containing less than $\frac{1}{2}$ of an eukaryotic viral genome propagated in cell culture (with the exception of DNA from Risk Group 3, 4 or restricted agents);
- 2.) rDNA work involving E.coli K12, S. cerevisiae, and B. subtilis hot-vector systems (with the exception of DNA from Risk Group 3, 4, or restricted agents).

All laboratories that work with human pathogenic material (human tissue and blood products) must register with the Yale Biological Safety Committee through the Form 01 Registration.

Appropriate forms for this registration can be obtained by calling the Occupational Health and Safety Section of the Office of Environmental Health and Safety at 737-2121.

The Form 01 and rDNA forms are also available in Acrobat PDF format from the OEHS Web site at: www.yale.edu/oehs/pdfforms.htm

Work with Recombinant DNA (rDNA)

SUMMARY:

Standard or Guideline:	Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)
Key P.I. Requirements:	Registration of non-exempt rDNA work Obtain approval for rDNA work before initiation Notify Yale BSC of major modifications of work Set policy for safe conduct of work in lab Train personnel in safe practices Report violations, accidents, illnesses to Yale BSC
Registration Form:	Yale BSC Registration of rDNA Experiments
Resources:	Laboratory Safety Monograph (available through the NIH Office of rDNA Activities, 301 496-9838).

OHS Biosafety can assist by reviewing proposed facilities, safety equipment, and work practices, and can conduct BL1, BL2, or BL3 training sessions for your research group.

Contact the OHS Biosafety Office at 785-3550 for a rDNA Registration Form, copies of the NIH Guidelines, or for further information. You can also access Biosafety information directly from our web site at <http://www.yale.edu/oehs/>

The *Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) outline the procedures required for use of rDNA, and describe the roles and responsibilities of the University and the principal investigator (P.I.). The University is responsible for ensuring that the rDNA activities comply with the provisions of the NIH Guidelines. (A complete description of the University's responsibilities can be found in Section IV-B of the NIH Guidelines).

Proposals for non-exempt rDNA work are submitted to the Yale Biological Safety Committee for review prior to initiation. The Committee is responsible for review of rDNA experiments for compliance and for assessing the containment level, facilities, procedures, practices, and expertise and training of research personnel. Committee results are communicated to the P.I. describing the containment level and any additional precautions. The Committee will also periodically review rDNA research at the University to ensure that the University is in compliance with the NIH Guidelines.

The P.I. is ultimately responsible for compliance with the NIH Guidelines and for the safe conduct of rDNA experiments. S/he must perform an initial risk assessment for rDNA work and identify an appropriate containment level for the experiment. In addition, the P.I. must ensure that all personnel involved in the experiment are trained in safe working procedures. (A complete list of P.I. responsibilities can be found in Section IV-B-7 of the NIH Guidelines). These responsibilities are also outlined on the rDNA registration form). Experiments that require Yale Biological Safety Committee approval may not be initiated or modified until approval has been obtained from Committee.

According to the NIH Guidelines recombinant DNA molecules are defined as either:

(1) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; or

(2) molecules that result from the replication of those described in (1) above.

Experiments involving rDNA are classified on the basis of hazard and fall into the three major categories highlighted in bold below:

(A) Experiments that Require Institutional Biosafety Committee Approval Prior to Initiation (Sections III-A, III-B, III-C, and III-D pages 11-15);

III-A-1-a: Deliberate Transfer of a drug trait to a microorganism not known to acquire it naturally;

III-B-1: Cloning of DNA encoding molecules lethal to vertebrates at an LD 50 of <100ug/kg body weight;

III-C-1: Human gene transfer experiments;

III-D-1: Cloning using human or animal pathogens as host-vector systems: Refer to the "Classification of Etiologic Agents on the Basis of Hazard" (from Appendix B of the NIH Guidelines). Please contact the Biosafety Office (785-3550) for assistance with risk assessment and for information on any agents that are not listed in the Classification;

III-D-2: Cloning of DNA from all Class 3, 4, or 5 human or animal pathogens (including HIV and related viruses, and human tumor viruses);

III-D-3: Experiments using more than 2/3 of the genome of infectious animal or plant viruses or defective viruses grown in the presence of helper virus;

III-D-4: Recombinant DNA experiments involving whole animals. Note that transgenic or knockout rodent experiments that require BL1 containment can be initiated simultaneously with Biosafety Committee notice. The purchase of transgenic or knockout rodents for BL1 experiments is exempt from the NIH Guidelines;

III-D-5: Recombinant DNA experiments involving whole plants;

III-D-6: Large scale DNA projects (>10 liters of culture).

(B) Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation (Section III-E, pages 15-17); and

III-E-1: Experiments using as vectors $\leq 2/3$ of the genome of an eukaryotic virus, free of helper virus;

III-E-2: Low Risk rDNA Plant Experiments;

III-E-3: Transgenic or knockout rodent experiments that require BL1 containment.

(C) Exempt Experiments - experiments that are of minimal hazard and do not require registration (Section III-E, page 17).

- ◆ rDNA containing less than 1/2 of an eukaryotic viral genome propagated in cell culture (with the exception of DNA from Class 3, 4 or 5 agents);
- ◆ rDNA work involving E. coli K12, S. cerevisiae, and B. subtilis host-vector systems (with the exception of DNA from Class 3, 4 or 5 agents); and
- ◆ The purchase or transfer of transgenic rodents for experiments that require BL1 containment.

Important Sections of the NIH Guidelines include:

Section II	Safety Considerations (Risk assessment and containment)
Section III	Experiments Covered by the NIH Guidelines (as described above)
Section IV	Roles and Responsibilities (Institution and PI)
Appendix B	Classification of Human Etiologic Agents on Basis of Hazard
Appendix G	Physical Containment (Biosafety Levels 1 –4)
Appendix H	Shipment
Appendix M	Points to Consider for Human Gene Transfer Experiments
Appendix P	Physical Containment for rDNA Plant Research
Appendix Q	Physical Containment for rDNA Research Involving Animals

Please refer to the NIH Guidelines for a complete list of contents.

NIH Guidelines on-line: <http://www.nih.gov/od/orda>

Appendix F Human Gene Transfer Clinical Trials

Proposed clinical trials involving human gene transfer require registration and approval from both campus and federal agencies before initiation. NIH defines human gene transfer as the “the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects.” The Yale University Institutional Biological Safety Committee requirements for human gene therapy protocols are detailed below. Federal requirements (NIH and FDA) for these experiments are described in significant detail in Appendix M of the NIH Guidelines for Research Involving Recombinant DNA, May, 1999, and in the Code of Federal Regulations, 21 CFR, Part 312 (FDA Points to Consider).

On Campus Registrations and Approvals:

Yale Human Investigation Committee (IRB) 785-4688
Yale Biological Safety Committee (IBC) 785-3550 (through Biosafety Representatives)

Yale New Haven Hospital Subcommittee on Safe Handling of Gene Transfer Products 688-4634
(YNHH Hospital Epidemiology and Infection Control)

Federal Registration and Approval:

NIH Office of Biotechnology Activities (301) 496-9838
<http://www4.od.nih.gov/oba/apndx.htm> Appendix M (Human Gene Therapy)

FDA Center for Biologics Evaluation and Research
<http://www.fda.gov/cber/ind/ind.htm> 21 CFR Part 312

Note: Approval from the Yale IRB and IBC are required prior to submission to the YNHH Subcommittee on Safe Handling of Gene Transfer Products, or the FDA. In accordance with the update in the *NIH Guidelines for Research Involving Recombinant DNA*, Appendix M, Yale University’s IRB and IBC cannot approve a HGT protocol until it has been reviewed by the NIH Recombinant DNA Advisory Committee. Upon notification from the NIH Office of Biotechnology Activities that the RAC has completed its review, the IRB and IBC will be eligible to complete their respective reviews and approve if warranted. The Yale Biological Safety Committee Human Gene Therapy Subcommittee is the primary review arm for the Yale Biological Safety Committee for protocols involving human gene transfer.

Appendix M-1 of the NIH Guidelines includes the submission requirements and addresses for both the NIH Office of Biotechnology Activities and FDA Center for Biologics Evaluation and Research. A copy of the 21 CFR, Part 312 detailing the FDA IND Content and Format requirements can be downloaded directly from the FDA web address listed above.

Application for Human Gene Transfer Clinical Trials at Yale

To initiate the review of a proposed human gene transfer clinical trial, please submit a description of your protocol in the format described in Appendix M of the attached NIH Guidelines for Research involving recombinant DNA, May, 1999. To obtain a copy of the NIH Guidelines, access the NIH OBA web site or contact the Biosafety Office at 785-3550. Send a copy of your completed Appendix M to:

The Yale Biological Safety Committee
C/O Biosafety Office
Yale Office of Environmental Health & Safety
135 College Street, 1st Floor
New Haven, CT 06510

Contact person: Biosafety Officer, 785-3550

Only complete protocols will be sent to Committee members for review. Specifically, we'll need:

- ◆ Scientific abstract
- ◆ Non-technical abstract
- ◆ Your responses to Appendix M-II through M-V
- ◆ Your response to Adverse Event reporting requirements detailed in Appendix M-VII
- ◆ A copy of your HIC clinical protocol (your IND Submission)
- ◆ A copy of the HIC approved Informed Consent Document
- ◆ Curricula vitae (2 pages) for each key professional in biographical sketch format
- ◆ The proposed location for vector production and description of the Good Manufacturing or Good Clinical Practices that will be utilized to prepare the vector
- ◆ A copy of the Certificate of Analysis (CoA) for sterility for each lot of vector made at Yale or sent to the University for this experiment

Additional responsibilities of the Principal Investigator conducting a rDNA experiment are detailed in Section IV-B-7, Roles and Responsibilities of the NIH Guidelines. The full set of PI responsibilities can be accessed at <http://www4.od.nih.gov/oba/sect4.htm>

Adverse Events

All adverse events must be reported in an annual data summary that is prepared for the Yale HIC, the Yale Biological Safety Committee Human Gene Therapy Subcommittee, the FDA, the NIH Office of Biotechnology Activities, and your sponsor. Any **Serious Adverse Events (SAE's)** must be reported by telephone within 24 hours followed by a written report within 10 days. This report must be on file with the Yale HIC, the Human Gene Therapy Subcommittee, the NIH OBA, the FDA, and NIH Office for Protection from Research Risks if applicable within 15 days. **Please note that SAE's must be reported whether related to the protocol or not.** SAE's shall not be designated as confidential, either in whole or in part, and the SAE reports shall be stripped of patient identifiers, such as name, address, contact information, social security numbers, and date of birth. If the SAE occurs after the trial and deemed related to the HGT trial, it must be reported within 15 days of the date of determination.

The NIH OBA reporting form can be downloaded from their website at <http://www.nih.gov/od/oba>.

Yale University Human Investigation Committee (HIC)

The Yale HIC must approve all experiments involving human subjects prior to initiation. Please contact the HIC at 785-4688 for information on their requirements.

Human Gene Transfer at Yale New Haven Hospital

For the Yale New Haven Hospital Subcommittee on Safe Handling of Gene Transfer Products, please contact the YNHH Hospital Epidemiology and Infection Control Department at 688-4634, well in advance of the proposed start date to initiate review of any human gene therapy work planned there.

Please don't hesitate to contact Biosafety at 785-3550 if you have any questions.

Appendix G Sources of Contamination

If contamination is experienced in the laboratory, the following items may be sources of the contamination. For additional assistance please contact the Biosafety Office at 785-3550.

- ◆ Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., do not belong in the laboratory. These articles should be stored elsewhere.
- ◆ Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens or supplies, and outside air is a complication that may affect or invalidate the results of an experiment. Human sources of this type of contamination are evaluated as follows:
 - Sneezing, coughing and talking. Sneezing, variously reported to generate as many as 32,000 or 1,000,000 droplets below 100 microns in diameter; coughing, which produces fewer and larger droplets; and talking, which has been reported to average only 250 droplets when speaking 100 words, show great differences between persons in regard to the number of microorganisms aerosolized. As a general rule, it may be said that these actions by normal healthy persons may play a less important role in transmission of airborne infection to humans or experimental materials than does liberation of microorganisms from human skin.
 - Dispersal of bacteria from human skin. There is a tremendous variation in the number of bacteria shed from the skin by a clothed subject. For instance, in one study, the number varied from 6,000 to 60,000 per minute. These bacteria were released on skin scales of a size that could penetrate the coarse fabric used for the laboratory and surgical clothing in the test. Dispersal of skin bacteria was several times greater from the area below the waist than from upper parts of the body. Effective reduction is accomplished by use of closely-woven or impervious clothing fitted tightly at the neck, wrists, and ankles to prevent the clothing from acting as bellows that disperses air carrying skin scales laden with bacteria. Such clothing sometimes is too warm to work in. The purpose of this summary is to alert laboratory personnel to the existence of this source of contamination.
 - Prolific dispersal of bacteria occurs from infected abrasions, small pustules, boils, and skin disease. Washing of lesions with germicidal soap will greatly decrease the number of organisms on the skin and dispersal into the air. Healthy nasal carriers who generate aerosolized staphylococci usually can be identified by the presence of heavy contamination of their fingers, face, and hair. This point may be useful in investigating the source of staphylococcal contamination of cell lines.
 - Footwear. In moderate and high-risk situations, shoes reserved only for laboratory use have been recommended as a precaution against transporting spilled infectious agents outside the laboratory. In experiments during which reduction of potential contamination of experimental materials is important, laboratory-only shoes can also reduce the microbial load brought into the laboratory each day by street shoes. Shoes are efficient transporters. In one study, there were 4 to 850 times as many bacteria per square centimeter on the laboratory footwear as on the floor itself.

Personal Work Practices

- ◆ Food, candy, gum, and beverages for human consumption will be stored and consumed only outside the laboratory.
- ◆ Smoking is not permitted in the laboratory or Yale University buildings.
- ◆ Shaving and brushing of teeth are not permitted in the laboratory. Razors, toothbrushes, toiletry supplies and cosmetics are permissible only in clean areas, and should never be used until after showering or thorough washing of the face and hands.

- ◆ A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination, because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate facial fit of a facemask or respirator when the work requires respiratory protection.
- ◆ Develop the habit of keeping hands away from mouth, nose, eyes, face and hair. This may prevent self-inoculation.
- ◆ For product protection, person with long hair should wear a suitable hair net or head cover that can be decontaminated. This has long been a requirement in hospital operating rooms and in facilities where biological pharmaceutical products are manufactured. A head cover also will protect the hair from fluids, splashes, from swinging into Bunsen burner flames and Petri dishes, as well as reduce facial contamination caused by habitual repetitive manual adjustment of the hair.
- ◆ Long flowing hair and loose flapping clothing are dangerous in the presence of open flame or moving machinery. Rings and wristwatches also are a mechanical hazard during operation of some types of machines.
- ◆ Contact lenses do not provide eye protection. The capillary space between the contact lenses and the cornea may trap any material present on the surface of the eye. Caustic chemicals trapped in this space cannot be washed off the surface of the cornea. If the material in the eye is painful or the contact lens is displaced, muscle spasms will make it very difficult, if not impossible, to remove the lens. For this reason, contact lenses must not be worn by persons exposed to caustic chemicals unless safety glasses with side shields, goggles or full face shield are worn to provide full protection.
- ◆ Plants, cut flowers, an aquarium, and pets of any kind are undesirable sources of yeast, molds and other potential microbial contaminants of biological experimental materials.
- ◆ Books and journals returnable to the institutional library should be used only in the clean areas as much as possible.
- ◆ When change rooms with showers are provided, the employer should furnish skin lotion.
- ◆ When employees are subject to potential occupational infection, the shower and/ or face/hand-washing facilities should be provided with germicidal soap.
- ◆ Personal cloth handkerchiefs should not be used in the laboratory. Disposable cleansing tissues should be available for use instead.
- ◆ Hand washing for personal protection:
 - This should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, or entry at the wrist.
 - Throughout the day, at intervals dictated by the nature of the work, the hands should be washed. Presence of a wristwatch discourages adequate washing of the wrist.
 - Hands should be washed after removing soiled protective clothing, before leaving the laboratory area, before eating and smoking. The provision of hand cream by the employer encourages this practice.
 - A disinfectant wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin.
- ◆ Anyone with a fresh or healing cut, abrasion, or skin lesion should not work with infectious materials unless the injured area is completely protected, such as with waterproof bandages and double gloving.
- ◆ Persons vaccinated for smallpox may shed vaccinia virus during the phase of cutaneous reaction. Therefore, vaccination requires permission of the appropriate supervisor, because two weeks absence may be necessary before returning to work with normal cell cultures or with susceptible animals, such as the normal mouse colony.

- ◆ Use of surgeon's mask of gauze or filter paper is of little value for personal respiratory protection. It is designed to prevent escape of droplets from the nose or mouth. If use of biohazards demands respiratory protection, contact the Biosafety Office for assistance.

Appendix H Biomedical Waste

A. Introduction

You play an important role in Yale's medical waste program if you generate waste in a laboratory or clinical area. This guide will help you dispose of your medical waste in an easy and legal manner.

Our program is designed to protect the people who handle, transport and dispose of your waste. The program is also designed to protect the environment and minimize Yale's regulatory liability.

Some people believe they can save money by working around this program. These attempts are counter-productive. They may place other people and the University at risk. The costs associated with one injury, or violation fines can easily exceed annual operational costs. We would much rather hear and consider your suggestions for program improvement than have you implement unauthorized procedures.

The Environmental Services Section is continually working behind the scenes to improve this program and to control its cost. Direct any questions or suggestions to the Medical Waste Supervisor at 785-7585. Call if you have questions about unusual situations or anything not covered in this guide.

Remember: Radioactive or hazardous chemical wastes shall be disposed of through the radioactive waste stream or the hazardous chemical waste stream respectively.

Please note: Clean broken or unbroken graduate cylinders, Erlenmeyer flasks, and beakers can be disposed of through the general trash. Place the items in a cardboard box, seal it, and label it "broken glass".

B. Medical Waste Management - Overview

All medical waste must be contained in a sealed:

- ◆ beige sharps container,
- ◆ red sharps container,
- ◆ or orange autoclave bag

You can minimize costs by filling these containers efficiently and following the instructions in this guide. Waste in all sharps containers and orange autoclave bags must be autoclaved or chemically disinfected before being placed in a box-bag unit. A box-bag unit consists of the white medical waste box and the intact red bag liner. Autoclaved containers and bags should be allowed to cool before being placed in a box-bag unit. Place all sharps containers and bags upright in the box-bag unit. This will help minimize leaks and spills during transport.

When the box-bag unit is full, seal the red bag, attach an address label (available at the stockrooms), seal the box and apply a second address label to the outside of the box signing off in the location provided to indicate that the waste is packaged appropriately.

Keep your box-bag units inside your laboratory. Compliance with fire codes and maintaining control of this special waste stream is very important. Call the Office of Environmental Health and Safety at 785-3551 to have the sealed box-bag units removed. At the same time, they will deliver an equal number of empty box bag units so you have a continuous supply.

Beige and red sharps containers, autoclave bags and box-bag units shall only be used for medical waste disposal.

C. Ordering Procedures

Extra box-bag units can be obtained from Custodial Services at no cost to your laboratory. Call 785-4757 in the medical school, and 432-2780 in the central and science areas.

Red sharps containers are delivered by the Environmental Services Section at no cost to your laboratory. Call 785-7585 to place your order.

Beige sharps containers are available at either the Medical School stockroom or Kline Biology Tower stockroom at no cost to your laboratory.

Orange autoclave bags may be purchased at either the Medical School stockroom or Kline Biology Tower stockroom.

D. Definition of Medical Waste

Medical wastes are defined using the following criteria:

1. Waste Cultures and Stocks of Microorganisms or Etiologic Agents Including:

- a. Cultures and stocks of infectious agents or microorganisms from facilities assigned to Biosafety Levels 1 through 3 (BL1, BL2, BL3).
- b. Cultures of specimens from medical and pathological laboratories.
- c. Disposable containers, materials, and supplies that may have been contaminated during the manipulation of microbial cultures and stocks
- d. Wastes from the production of biologicals (including all tissue culture materials.)
- e. Live and attenuated vaccines.

2. Human Pathological Wastes

Pathological waste consists of human tissues; organs; body parts; blood; dialysate; cerebrospinal, synovial, pleural, peritoneal, and pericardial fluids; and their respective containers.

3. Waste Human Blood and Blood Products and Their Containers Including:

- a. Waste human blood and blood products (e.g. blood plasma, platelets, red or white corpuscles, and other derived licensed products such as interferon, etc.)
- b. Items saturated or dripping with human blood or blood products.
- c. Items caked with dried human blood or blood products.
- d. Intravenous bags.

4. Used Sharps Waste

This category includes used hypodermic needles, syringes (with or without the attached needles), pasteur pipettes, disposable plastic pipettes, scalpel blades, razor blades, blood vials, test tubes, needles with attached tubing, broken plastic culture dishes, unbroken glass culture dishes, and other types of broken and unbroken glassware that were in contact with infectious material including microscope slides and coverslips.

5. Unused Sharps Waste

Unused hypodermic needles, suture needles, syringes, and scalpel blades.

6. Waste Animal Carcasses, Body Parts, and Bedding

Animal wastes purposely infected or known to have been exposed to Class 1, 2 or 3 agents shall be autoclaved. Use the pre-existing disposal system implemented by the Animal Resource Center.

Uninfected or "clean" animals shall be wrapped in a nondescript plastic bag and discarded into grey barrels in Animal Resource Center coolers. Do not wrap these animals in orange autoclave bags or red bags. Plastic garbage bags can be purchased at either the Medical School stockroom or Kline Biology Tower stockroom.

7. Isolation Wastes

Isolation wastes are defined as biological wastes and discarded materials contaminated with blood, excretion, exudates, or secretions from humans or animals isolated due to infection with Class 4 microbial agents.

If a human or animal is known to be infected with a Class 4 agent, contact the Biological Safety Officer (737-5009) immediately.

E. Look-Alike Waste

Look-alike waste is not considered medical waste. Look-alike waste is plastic or glass labware, lab matting and gloves that have not been in contact with infectious material. Look-alike waste is disposed of through a separate waste stream and should not be placed in the medical waste stream. Items should be discarded in a manner to prevent physical injury to those people handling the waste. Glass and items that are capable of puncturing bags should be placed in a plastic lined cardboard box. Do not autoclave or chemically decontaminate look-alike waste.

All intravascular sharps are considered medical waste regardless of the presence of infectious material and must be discarded in beige sharps containers. Do not discard intravascular sharps in the look alike waste stream.

F. Disposal Procedures

1. Sanitary Sewer

The sanitary sewer was designed for the disposal of certain liquid wastes. Use of the sanitary sewer reduces the chance for leaks or spills during transport and reduces disposal costs.

- Waste microbiological liquid stocks (Class 1,2 and 3 agents) shall be autoclaved or chemically disinfected and poured down the drain whenever possible.
- Human blood and body fluids do not need to be disinfected before being poured down the drain.
- Remember to rinse the sink area afterward. Disinfect if necessary.

2. Beige Sharps Containers

- Discard all intravascular sharps waste such as hypodermic needles, syringes (with/without the attached needles), scalpel blades, and suture needles in your beige container.
- You may also deposit any other type of sharps waste into this container.
- Autoclave or chemically decontaminate waste in the container. Place the decontaminated and drained container upright into your box-bag unit.

3. Red Sharps Containers

Do not discard needles, syringes or other intravascular sharps into a red bucket. It is illegal to do so.

- Discard all non-intravascular sharps waste such as: pasteur pipettes, disposable plastic pipettes, blood vials, test tubes, glass culture dishes, microscope slides and overslips, sharp broken plasticware and other types of broken or unbroken glassware that may have been in contact with infectious material.
- Autoclave or chemically decontaminate waste in the container. Place the decontaminated and drained container upright into a box-bag unit.

4. Orange Autoclave Bags

- Place small volume pathological waste, empty intact plastic liquid waste containers (with a residual volume of less than 20 cubic centimeters); intact plastic blood containers (with a residual volume of less than 20 cubic centimeters); intact plastic disposable containers; all other non-sharp materials and supplies that may have been contaminated during the manipulation of microbial cultures and stocks; and non-sharp waste from the production of biologicals (including tissue culture materials) in the orange autoclave bag.
- Autoclave the orange bag before placing it upright in a box-bag unit.

5. Medical Waste Box-Bag Unit

- Place your decontaminated beige sharps containers, red sharps containers and orange autoclave bags upright into a box-bag unit

G. Biological Decontamination Procedures

Sharps containers and orange autoclave bags shall be biologically decontaminated before being placed in a box-bag unit.

1. Bleach Decontamination:

Fill the sharps container with a 1:10 bleach dilution and allow to stand overnight. Invert the container in a sink and drain off the bleach solution.

2. Autoclaving is preferred:

Do not autoclave hazardous chemicals. When autoclaving a red sharps container leave the cover open. Beige sharps containers can be closed before being autoclaved. Orange autoclave bags should not be taped closed. State regulation requires autoclaves to be operated at 250°F and 15 pounds per square inch pressure for 60 minutes. Drain excess liquid and allow to cool before placing container into a box-bag unit.

H. Mixed Waste

1. Chemical Waste and Medical Waste

Items contaminated with ethidium bromide, diaminobenzidine (DAB), phorbol, or phenol-chloroform mixtures should not be mixed with other medical waste. Segregate these items into a beige sharps container, red sharps container or orange autoclave bag and label accordingly. When the container is full, place it directly into a box-bag unit. Do not autoclave or bleach decontaminate.

2. Chemotherapy Waste and Medical Waste

Items contaminated with trace amounts of a chemotherapeutic agent or empty stock bottles may be disposed of through the medical waste stream. "Empty" is defined as containing less than 3% by weight of the total capacity of the container.

Stock solutions of these chemicals and items that are heavily contaminated are disposed of through the Chemical Hazardous Waste Program.

Call the Environmental Services Section (785-3551) for guidelines concerning the disposal of chemical hazardous waste.

3. Radioactive Waste and Medical Waste

Radioactive sharps waste should be disposed of in the yellow sharps containers provided by the Environmental Services Section.

Animal carcasses, tissue/parts, and excreta containing/contaminated with radioactive materials shall be disposed of according to Environmental Services Section requirements.

4. Other Mixed Waste Issues

If you have any questions regarding other mixed waste issues please call the Environmental Services Section (785-3551).

I. Beige Sharps Containers

General Procedures

- ◆ All non-radioactive hypodermic needles, syringes (with or without the attached needles), and other intravascular sharps waste must be discarded into beige containers.
- ◆ Other non-radioactive sharps waste that was in contact with infectious agents or other biologicals may be deposited into beige containers.
- ◆ Containers may be used freestanding or wall mounted. Order containers and wall brackets through the Environmental Services Section (785-7585).
- ◆ In patient-care settings, containers shall be locked to wall brackets.
- ◆ Non-radioactive sharps waste shall be deposited into the opening on the top of the container (The flap on top of the container is a lid - not a flipper).
- ◆ The clear top allows you to see when the container is full.
- ◆ Do not overfill container.
- ◆ Sharps containers shall not be left in hallways, stairwells, or used as door stops.

Biological Decontamination

All sharps containers shall be biologically decontaminated.

CHEMICAL DECONTAMINATION: Sharps contaminated with ethidium bromide, diaminobenzidine (DAB), phorbol or phenol-chloroform mixtures are already chemically decontaminated. Segregate these sharps into one container and label accordingly. Do not autoclave or bleach.

BLEACH DECONTAMINATION: Fill the container with a 1:10 dilution of bleach. Push the flap on top of the container into the opening and past the first or second set of stops. Allow the bleach solution to stand in the container overnight. Invert the container in a sink to drain off the bleach solution.

AUTOCLAVING IS PREFERRED: (Hazardous chemicals shall not be autoclaved). Push the flap on top of the container into the opening and past the first or second set of stops. Autoclave the container at 250°F and 15 pounds per square inch of pressure for 60 minutes.

Disposal

After biological decontamination, place container upright into Medical Waste box-bag unit. When the box-bag unit is full, call the Environmental Services Section at 785-7585 for a waste inspection.

J. Red Sharps Containers

General Procedures

- ◆ Non-radioactive, non-intravascular sharps shall be deposited into a red sharps container, including:
 - pipettes
 - blood vials
 - glass test tubes
 - microscope slides and coverslips
 - glassware that has been in contact with biological materials
 - broken plasticware
- ◆ Hypodermic needles, syringes (with or without their attached needles), and other intravascular sharps must be discarded into a beige sharps container.
- ◆ Do not overfill container.
- ◆ Sharps containers shall not be left in hallways, stairwells, or used as door stops.
- ◆ Order red sharps containers through the Environmental Services Section (785-7585).

Biological Decontamination

All sharps containers shall be biologically decontaminated.

CHEMICAL DECONTAMINATION: Sharps contaminated with ethidium bromide, diaminobenzidine (DAB), phorbol or phenol-chloroform mixtures are already chemically decontaminated. Segregate these sharps into one container and label accordingly. Do not autoclave or bleach.

BLEACH DECONTAMINATION: Fill the container with a 1: 10 dilution of bleach, close the lid, and allow to stand overnight. Invert the container in a sink and drain off the bleach solution.

AUTOCLAVING IS PREFERRED: (Hazardous chemicals shall not be autoclaved). With the cover partially open, autoclave the container at 250°F and 15 pounds per square inch of pressure for 60 minutes.

Disposal

After biological decontamination, place the container upright into a Medical Waste box-bag unit. When the box-bag unit is full, call the Environmental Services Section at 785-7585 for a waste inspection.

Posters

BL1 Laboratory Practices

1. Keep laboratory door closed when experiments are in progress.
 2. Use procedures that minimize aerosols.
 3. Do not smoke, eat, drink or store food in BL1 areas.
 4. Wear laboratory gowns or coats when appropriate.
 5. Do not mouth pipette. Always use mechanical pipetting devices.
 6. Avoid using hypodermic needles.
 7. Wash hands after completing experimental procedures and before leaving laboratory.
 8. Disinfect work surfaces daily and immediately after a spill.
 9. Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
 10. For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
 11. Control insect and rodent infestations.
 12. Keep areas neat and clean.
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BL2 Laboratory Practices

1. Keep laboratory door closed.
 2. Post a universal biohazard label on equipment where infectious agents are used/stored.
 3. Allow only persons informed of the research to enter BL2 areas.
 4. Keep animals not used in BL2 experiment out of the laboratory.
 5. Do not smoke, eat, drink, or store food in BL2 areas.
 6. When appropriate, wear laboratory gowns or coats.
 7. Do not mouth pipette. Always use mechanical pipetting devices.
 8. Use procedures that minimize aerosol formation.
 9. Avoid using hypodermic needles.
 10. Use biological safety cabinets to contain aerosol-producing equipment.
 11. Wash hands after completing experimental procedures and before leaving laboratory.
 12. Disinfect work surfaces daily and immediately after a spill.
 13. Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
 14. For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
 15. Control insect and rodent infestations.
 16. Keep areas neat and clean.
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Centrifuge Safety

- ◆ Each operator must be trained on the proper operating procedures
- ◆ Keep a log book detailing operation records for centrifuges and rotors
- ◆ Do not exceed safe rotor speed
- ◆ Place a biohazard label on the centrifuge if used for infectious agents
- ◆ Always use sealed safety buckets or sealed rotors with O-rings
- ◆ Load and unload safety buckets or rotors within the biosafety cabinet
- ◆ Check tubes and bottles for cracks and deformities before each use
- ◆ Examine O-ring and replace if worn, cracking or missing
- ◆ Never overfill primary containers; do not exceed $\frac{3}{4}$ full
- ◆ Wipe exterior of tubes or bottles with disinfectant prior to loading into safety buckets or rotor
- ◆ Wipe the exterior of safety buckets or rotors with disinfectant before removing from biosafety cabinet
- ◆ Stop the centrifuge immediately if an unusual condition, such as noise or vibration, begins
- ◆ Wait five minutes after the run before opening the centrifuge to allow aerosols to settle in the event of a breakdown in containment
- ◆ Decontaminate safety buckets or rotors and centrifuge interior after each use
- ◆ Wash hands after removing gloves

Centrifuge Spill

If you notice that there has been a leak outside the safety bucket or rotor when opening centrifuge:

First:

- ◆ Hold Breath
- ◆ Close centrifuge lid
- ◆ Notify others to evacuate the lab

Then:

- ◆ Immediately leave the lab
- ◆ Post biohazard spill sign

Notify PI or Supervisor:

- ◆ DO NOT re-enter lab until PI and OEHS have given clearance (at least 30 minutes)
- ◆ Follow centrifuge spill instructions in the Biosafety Manual or Spill Response Guide

Decontaminate:

- ◆ Remove PPE turning exposed areas inward
- ◆ Place disposable PPE in biomedical waste (autoclave reusable PPE)
- ◆ Wash any exposed areas with antiseptic soap and water
- ◆ Wash hands thoroughly

For Centrifuge Explosion:

Evacuate room immediately; notify PI and OEHS

Autoclave Safety

Steam sterilization has been an indispensable tool in biological research since Pasteur's time. Despite this importance, many people are unaware of some basic autoclave operating procedures that can improve the quality of sterilization as well as reduce the risk of personal injury.

- ◆ Never autoclave nitrocellulose tubes – they can explode!
 - ◆ Carefully prepare items for autoclaving. Loosely cover or cap containers to avoid over-pressurization.
 - ◆ Keep loads small – overloading hinders steam penetration.
 - ◆ Bags should be left partially open and should be contained within a tray.
 - ◆ If time allows let the load cool before removing it from the autoclave. Otherwise, open the door about ½ inch and vent for 5-10 minutes before emptying autoclave.
 - ◆ Wear shoes/sneakers, pants, lab coat, face shield, and long sleeved insulated gloves when operating an autoclave. A heavy, rubberized insulated apron is further recommended for those who autoclave frequently.
 - ◆ Periodically verify autoclave effectiveness with biological and chemical indicators that are available from the Biosafety Office.
 - ◆ If you experience any problems or unusual occurrences please report them to your supervisor or manager, Building Operations Coordinator, or the Office of Environmental Health & Safety (785-3550).
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Toxins

Safe Working practices to minimize exposure via ingestion, inhalation, mucous membrane contact, and absorption or penetration through the skin.

BL2 Work Practices

- ◆ Label toxin work areas within lab
- ◆ Cover work surface with plastic-backed absorbent paper
- ◆ Avoid generating aerosols; handle the powdered form carefully
- ◆ Use a chemical fume hood or biosafety cabinet when feasible
- ◆ Avoid the use of needles or Pasteur pipettes
- ◆ Substitute plastic for glass wherever possible
- ◆ Decontaminate work surfaces with 5-10% household bleach or 0.1N sodium hydroxide
- ◆ Treat liquid waste with 50% household bleach (soak overnight.)
For T-2 mycotoxin use a combination of 50% household bleach and 0.25N sodium hydroxide.
- ◆ Collect and autoclave waste at the end of the day
- ◆ Autoclave or chemically disinfect contaminated protective clothing before reuse

Personal Hygiene

- ◆ Keep your hands away from your face
- ◆ Do not eat, drink, or smoke in the lab
- ◆ Do not mouth pipette
- ◆ Always wash hands after removing protective clothing and before leaving the lab

Labels and Transport

- ◆ Post BL2 biohazard sign at lab entry
- ◆ Restrict access to the lab
- ◆ Label equipment used with or storing toxins
- ◆ For transport, use sealed, unbreakable, leakproof containers with a biohazard label and full toxin name

Protective Clothing Requirements

- ◆ Lab coat buttoned to the top with knit or grip cuffs, or use gloves that are long enough to cover the sleeves; a back-fastening gown is suitable; sleeve covers offer additional protection
- ◆ Gloves (consider double gloving)
- ◆ Face protection such as a face shield or safety glasses and a mask to cover the eyes, nose and mouth
- ◆ Dedicate protective clothing for work with toxins and do not wear outside the lab
- ◆ Avoid skin contact when removing gloves

Work with Powdered Form of Toxin

- ◆ Carefully weigh and convert to aqueous form as soon as possible
- ◆ Store powdered form in an unbreakable secondary container labeled with the complete toxin name to identify the hazard
- ◆ Change gloves after handling powdered toxin being sure to avoid skin contact with the toxin while removing gloves; wash hands prior to donning new gloves

Emergency Response

- ◆ Flush skin or eyes with running water for 15 minutes, notify PI immediately, seek medical assistance
- ◆ Follow BL2 spill procedures: leave lab for 30 minutes, upon return, decontaminate spill with 25% household bleach solution for 30 minutes, collect and autoclave waste

Table of Principal Investigator Requirements

	Required Registration									Required Training					Required Inspection					
	Form 01 and annual update	rDNA	Biological Safety Committee	State of Connecticut	Yale Animal Resource Center	Yale Animal Care and Use Committee	Yale HIC, FDA, NIH and YNH	CDC	USDA	Bloodborne Pathogens	Biosafety	Biosafety Level 3	YARC	Shipping & Transport of infectious Agents	Annual Biological/Chemical	Biosafety	CT Dept. of Public Health	CDC Select Agent	USDA	Infection Control (Patient Areas)
Human Materials and cell lines of human origin	X		X							X					X					
Recombinant DNA – exempt	X										X				X					
Recombinant DNA – non exempt	X	X	X								X				X	X				
Infectious Agents – BL2	X		X	X							X				X	X	X			
Infectious Agents – BL2+ and BL3	X		X	X								X			X	X	X			
Animal Use	X				X	X							X		X					
Animal use with BL2 agent	X		X		X	X					X		X		X	X				
Animal use with BL2+ or BL3 agent	X		X		X	X					X	X	X		X	X				
Human Gene Therapy	X	X	X				X			X	X				X					X
Select Agents – See list in Appendix C	X		X					X			X				X	X		X		
USDA Regulated Agents	X		X						X						X	X			X	
Shipping Infectious Materials														X						

