Yale University
Environmental Health & Safety Office

Biological Safety Manual

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

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Exposure Incident

Report exposure immediately; you may need immediate therapy.

Needle sticks/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 Yale Health, Acute Care (432-0123). Medical Area employees may also go to the Y-NHH Emergency Room (688-2222) from 4:30 p.m. to 7:30 a.m. or Y-NHH Occupational Health Services, 688-2462, East Pavilion 1 - Room 40 (behind cafeteria) Mon. - Fri. excluding holidays 7:30 a.m. - 4:00 p.m.

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

Emergency Phone Numbers

Police and Fire: 911

Yale Health: 432-0123

EHS Emergency Numbers

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

Other hours: 911

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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 2016 *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 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://ehs.yale.edu). 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 Environmental 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,

Craig Russell Roy, PhD.
Chairman, Yale Biological Safety Committee
Department of Microbial Pathogenesis

1 Introduction

1.1 Emergency Phone Numbers and Environmental Health and Safety Contacts

Emergency Telephone Numbers

Ambulance/Fire/Police 911
Yale Health (203) 432-0123
Biological/Chemical/Radiological Emergencies (203) 785-3555 (Monday - Friday, 8:30 AM to 5:00 PM, at other times call Campus Police at 911)

Environmental Health and Safety Office Telephone Numbers

Office of Environmental Health and Safety	(203) 785-3550
Benjamin Fontes, Biosafety Officer	(203) 737-5009
Deborah Ferry, Associate Biosafety Officer	(203) 737-2125
Linda Mouning, Research Support Coordinator	(203) 737-2121

Environmental Health and Safety Office Web Site

http://ehs.yale.edu/

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 BSL2 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). Yale Biosafety Committee approval is required prior to initiation of any studies or research involving human or animal pathogens. The procedures for handling unclassified agents must also be reviewed by the Yale Biological Safety Committee and the Environmental Health and Safety Office (EHS), 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 EHS.

- 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 EHS Biosafety Office (Biosafety Office).
- Registering research work involving non-exempt recombinant DNA with the Biological Safety Committee. The Principal Investigator must complete the "Registration of Research Involving Recombinant or Synthetic Nucleic Acid Molecules" 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 for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).
- Below is a table outlining the registration and training requirements for which the Principal Investigator is responsible as well as the inspections that are required based on the materials being used.

1.2.2.1 Table of Principal Investigator Requirements

If using items below requirements indicated with an "X" must followed	Required Registration									Required Training				Required Inspection					
	Biological General and annual update	rDNA	Biological Safety Committee	State of Connecticut	Yale Animal Resource Center	Yale Animal Care and Use Committee	Yale HRPP, FDA and YNHH	CDC	USDA	Bloodborne Pathogens	Biosafety	Biosafety Level 3	YARC	Annual Biological/Chemical	Biosafety	CT Dept. of Public Health	CDC Select Agent	USDA	Infection Control (Patient Areas)
Human Materials and Human Origin Cell Lines	X		X							X				X					
Recombinant and Synthetic Nucleic Acid Molecules –exempt	X										X			X					
Recombinant and Synthetic Nucleic Acid Molecules – non-exempt	X	X	X								X			X	X				
Infectious Agents – BSL2	X		X	X							X			X	X	X			
Infectious Agents – BSL2+ and BL3	X		X	X								X		X	X	X			
Animal Use	X				X	X							X	X					
Animal Use with BSL2 agent	X		X		X	X					X		X	X	X				
Animal Use with BSL2+ or BL3 agent	X		X		X	X					X	X	X	X	X				
Human Gene Transfer	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	

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 regulations, guidelines, and standards. This includes completing the applicable EHS trainings and receiving laboratory specific training from the PI or the PI's designee.
- Follow the recommendations and requirements provided in 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 materials in use and emergency procedures to be followed in the event of an incident (e.g. exposure, spill).
- 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 and requires the cooperation of all facility users. The items below are some of the things that need to be done regularly and are often assigned to specific research staff on a rotating schedule or as a permanent responsibility:
  - Training: providing laboratory specific training to new staff members at the request of the PI.
  - Autoclaves and waste: discarding biomedical waste containers before they become overfilled.
  - Freezers: periodically defrost freezers. This is a good time to review the contents of cold storage devices to identify unknown materials and discuss the disposition of these materials with the PI.
  - Cleaning: regular cleaning and organization of individual work areas and common use areas.
  - Vacuum trap and filter maintenance: schedule the cleaning of vacuum collection flasks and ensure that the flasks are labeled with the disinfectant they contain and that there is an in-line vacuum filter in each vacuum trap setup.
  - Maintenance of supplies, including personal protective equipment (PPE).
  - Security of infectious agents; i.e. store infectious agents in a locked freezer in a locked laboratory.

1.2.4 Environmental Health and Safety Office

The Environmental Health and Safety Office (EHS) provides:

Information on laboratory operation to ensure compliance with CDC, NIH, OSHA and other applicable regulations, standards, and guidelines as well as Yale policies.

- Updates on regulations that apply to the laboratory and information about new regulations and how they impact the research being done.
- 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, standards, and Yale policies.

Biological Safety Officer:

The Biological Safety Officer (BSO) is responsible for the implementation of policy guidelines recommended by the Yale Biological Safety Committee (Yale BSC). The BSO identifies potential problem areas and suggests to the Yale BSC 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 EHS 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 or Synthetic Nucleic Acid 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.
- Providing various trainings related to biological safety and related topics.

1.2.5 Yale Biological Safety Committee

The Yale Biological Safety Committee (the Committee) shall serve as the Institutional Biosafety Committee (IBC) as defined in the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).* As such, the Committee shall review applications for research involving recombinant DNA (rDNA) to determine whether the facilities, procedures, and practices meet the standards required by the university and the NIH. The Committee provides annual certification to the NIH that facilities, procedures, and practices being used, 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 as required by the NIH Guidelines.

The committee's responsibilities include:

Reviewing rDNA and infectious agent registrations to ensure the PI understands the risks of the material to be used, has performed a complete risk assessment, and has specified the use of containment equipment, practices, and procedures to address the risks of the material.

Approving registrations that provide the appropriate level of protection to personnel working on the project described in the registration, other staff members in the area, and the environment.

- 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 EHS Director on policy matters concerned with the protection of personnel from biohazardous agents and materials that may be present in the laboratory 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.

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 with applicable regulations, standards, and Yale policies.

2.1 Biological General Registration

All Principal Investigators are required to complete and submit a Biological General Registration through Integrator. 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. EHS 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 Biological General Registration 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 or Synthetic Nucleic Acid 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 Biological General Registration is accessible at https://ehsis.yale.edu/EHSIntegrator/Registration

2.2 Human Pathogens

Prior to initiating work with a human etiologic agent, approvals are required from the:

- Yale Biosafety Committee
- State of Connecticut Department of Public Health (DPH)

To initiate these approvals, complete and submit an Infectious Agent Registration available at https://ehsis.yale.edu/EHSIntegrator/Registration. EHS staff will do a preliminary review of the registration. Once EHS staff approve the registration a member of the Biosafety Office presents the registration to the Yale Biosafety Committee for review and a vote to approve the registration.

Biosafety Office staff will then notify the DPH and arrange for inspectors from the DPH to inspect the labs listed in the registration where work with human pathogens will be performed.

In addition to the approvals above, experiments involving the introduction of infectious agents or potentially hazardous biological materials into animals also requires approval from the:

- Yale Institutional Animal Care & Use Committee (IACUC)
- Yale Animal Resources Center

Many etiologic agents have been assigned a risk group level based on the degree of hazard they pose. Risk groups (RG) are numbered 1-4 with RG1 being the lowest hazard and RG4 being the highest hazard. Work with RG4 agents (e.g. lassa, sabia, and hendra viruses, is not permitted at Yale or in Connecticut. Risk Group 2 and higher etiologic agents are listed in the NIH Guidelines. The NIH guidelines are available at the following web address: https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/. The list has been copied to Appendix B (*Classification of Human Etiologic Agents on the Basis of Hazard*) of this manual. Call the Biosafety Office for assistance with agents that are not listed.

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 available online at https://ehs.yale.edu/biological-trainings. The Principal Investigator is responsible for ensuring that all researchers are trained in the appropriate procedures and techniques used in the laboratory.

Staff members from EHS will periodically monitor your facility and procedures as well as answer any questions regarding biosafety.

Contact a Biosafety Office staff member before:

- Work with a new infectious agent is initiated
- Changing the scope or location of existing work
- Adding researchers from a registration
- 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 training and documentation; contact a staff member of the Biosafety Office for additional information.

2.3 Recombinant DNA Experiments

Yale Biological Safety Committee approval is required prior to the initiation of non-exempt recombinant DNA experiments. A brief description of non-exempt and exempt recombinant DNA experiments is provided in the Biosafety Office document Overview of Recombinant DNA Experiments Covered by The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) April 2019 available on the EHS web site at https://ehs.yale.edu/sites/default/files/files/rdna-experiments-nih.pdf.

Submit recombinant DNA registrations through EHS Integrator at https://ehsis.yale.edu/EHSIntegrator/Registration

Contact a Biosafety Office staff member to:

- Register non-exempt recombinant DNA work
- Update current registration if the scope of the work has changed
- Add a new researcher(s) to the registration
- Ask any questions regarding recombinant DNA work.

The Recombinant DNA Registration form is in Appendix E and is also on the EHS web site. The NIH guidelines are available at the following web address: https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/.

2.4 Human Gene Transfer (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 exposed to human blood or other potentially infected materials. Employees with a reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of their duties is considered occupationally exposed. Other potentially infectious materials include:

- Human cell or tissue cultures (primary and established)
- 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. A risk assessment must be completed to confirm the potential for exposure in these cases.

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

Consult the *Bloodborne Pathogen Training Manual* for additional information. The manual is available on the EHS web site at https://ehs.yale.edu/sites/default/files/files/bbp-training-manual.pdf. 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 Institutional Animal Care and Use Committee (IACUC) 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 IACUC protocol. Contact the IACUC at (203) 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 (203) 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 the IACUC at (203) 785-5992 or YARC at (203) 785-2526.

2.7 Biosafety 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.

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 on the certification/service contract will be placed in storage status. EHS 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 (e.g., replacing fluorescent lamps, switches, etc.) are needed 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 the Yale University Procurement 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. If you are using a LFB, 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 EHS 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 research at Yale University. Please review the following table for information on required training. You can access online EHS trainings at https://ehs.yale.edu/biological-trainings 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, human cell lines and bloodborne pathogens.	Bloodborne Pathogen Training for Laboratory Personnel: • Required before initiation of work and • At least annually thereafter.	Web based training or self-study guide
Human, animal or plant pathogens classified at BSL2	Biosafety Training Part I, and Biological Safety Training: Part II	Web based training
Human, animal or plant pathogens classified at BL3	Biosafety Level 3 Training	Call Biosafety at 785-3550 to schedule a class
An aspect of shipping biological materials (infectious agents, genetic material, and human or animal specimens)	Training requirements vary on the material being shipped. Please refer to the EHS web site at http://ehs.yale.edu/research-materials-shipping-training-requirements	Web based training
Contact with patients in a clinical setting	TB Exposure Control Training Bloodborne Pathogen Training for Clinical Personnel	Web based training Web based training

3 Medical Surveillance Program

A medical surveillance program of University personnel engaged in biological research is conducted by Employee Health at 55 Lock Street. 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 examination or 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 present 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 (203) 432-7978.

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.

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 8-10 weeks post exposure. Please contact Employee Health at (203) 432-7978 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 should 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.
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 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 to make recommendations to the respective supervisors or Principal Investigators.

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)
- 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 known reproductive hazard (generally with actual pathogens, not necessarily for only other potentially infectious materials such as blood or body fluids) will be discussed and can be reviewed per university human resources policies. Also, Investigators actively working with reproductive hazards are to 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 immunosuppressive conditions and drug therapies that suppress 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, which includes collection of pre-assignment serum. Additional serum samples will be collected If an illness occurs which may be related to the agent the person is working with.

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 BSL2 contamination (i.e. centrifuge incident) or incidents involving BSL2+ or BL3 agents, EHS 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 Acute Care 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 Yale Health. These records are kept by the Medical Records Department, 55 Lock Street, (203) 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 Yale Health, 55 Lock Street. Please also report incidents that did not result in an exposure (near miss) to EHS. 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 and the use of containment equipment 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 six P's of risk assessment and risk management are:

Pathogen – hazardous biological agent.

Procedures – proposed experimental manipulations

Personnel – appropriate training and skills.

Practices – significant work practices

Protective equipment – protective clothing and safety equipment.

Place – laboratory design.

Further information on the 6 P's of Risk Assessment and Risk Management are discussed here.

Risk Assessment

PATHOGEN review

Review of all proposed PROCEDURES with the biohazard

PERSONNEL evaluation

Risk Management

Formulation of work PRACTICES to mitigate identified risks

Selection of PROTECTIVE EQUIPMENT to place barriers between staff and the biohazard

Utilization of a PLACE or lab that is suitable for the proposed research

Step 1:
Pathogen (Gather ALL information on the biohazard or agent)

- Download the Pathogen Safety Data Sheet from https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

- Obtain the Agent Summary Statement from the CDC/NIH BMBL if available (https://www.cdc.gov/labs/BMBL.html)
- Understand the signs and symptoms of infection
- Learn preventive therapies (immunizations and post-exposure treatment)
- If a pathogen is antibiotic resistant or resistant to a commonly used therapy, know which treatments will be effective against the pathogen; this is especially important in the event of an exposure
- Know all clinical syndromes that can be caused by the agent
- Identify what medical conditions may make a person more susceptible or at higher risk of infection or serious disease
- Let staff know to seek counsel from Employee Health prior to starting work with biohazards for a private consult regarding their health status
- Identify the incubation period, the infectious dose, the starting Risk Group
- Do a web search for laboratory-acquired infections with the proposed biohazard and identify how it was transmitted if known
- Learn and understand how the biohazard is transmitted in nature and in a lab setting
- Review unnatural exposure routes with staff, including aerosol deposition to lower lung, aerosol contamination of mucous membranes, aerosol contamination of surfaces, hand to face (eyes, nose, and mouth) transfer of pathogens, eye to nasal cavity to back of throat to gut transmission
- Find out how long the biohazard can survive on surfaces or the environment
- List the disinfectants that are effective at inactivating the biohazard and identify the concentration of the disinfectant and contact time required for kill
- Highlight pertinent risk assessment information and share with your staff (list at start of site-specific standard operating procedures)
- Post unique risk information on the lab door biohazard sign to ensure all visitors are informed of potential risks

Step 2:
Procedures (review all proposed procedures with the biohazard or agent)

- Identify and list all the procedures, equipment and supplies that will be used in your research or lab protocol
- Ensure that every step is included, from removal of the biohazard from the freezer, transport to work areas, all protocol steps, through decontamination and disinfection and/or return to frozen storage
- Identify any steps performed outside your lab, such as in core facilities (i.e. specialized microscopy, flow cytometry) and any shared equipment locations
- Once all the steps, supplies and equipment have been documented and written down, identify all the risks and potential exposures that could possibly occur during the course of the work (splashes, splatter, spills, aerosols, cuts, lacerations, punctures, bites, scratches, etc.)
    - Pay particular attention to punctures from contaminated sharps (if not working with animals – eliminate sharps and use plastic alternatives!)
    - Any step with a liquid can generate splash or splatter that could contact facial mucous membranes, skin, or personal clothing and contact surrounding work areas
    - Procedures that impart energy to a culture (basically all of them) from pipetting to vortexing to centrifugation and highlight these steps that may produce aerosols
- For animal experiments consider the use of sharps for inoculation, bites and scratches from the animal, exposure to bedding contaminated from excretion of the biohazard
- Identify the potential for spills (dropped flasks, broken flasks in shakers, leaks in centrifuges, etc.)
- Write down all the steps and risks identified and detail the potential for exposure (this is your required written site-specific risk assessment!)

PAUSE: ask your Biosafety Officer or assigned Safety Advisor to review your risk assessment at this point. Also ask your IACUC, IBC or IRB rep, where applicable, to review. Your biosafety officer will confirm your risk assessment and help identify any procedures with potential risk that may have been missed. Update your written risk assessment after this review.

Step 3:
Personnel Evaluation (review who will be handling biohazards)

- Do all staff have prior experience working with this biohazard or very similar biohazards? If not, an internship to gain hands-on experience with the agent can be arranged with another lab or within your lab
- Have all staff completed all required Biosafety and other applicable laboratory safety trainings prior to initiating work?
- Do staff have a positive safety attitude and a healthy amount of respect for the risks involved with the proposed biohazard?
- Have the proposed staff exhibited a strong safety record in the lab?
- Are all staff informed of the risks presented by this work and the proposed procedures?
    - Are any staff at greater risk due to their health status?
        - Have they met with and been cleared by Employee Health?
            - This discussion of likely elevated risks and review of proposed participation is critical
    - Do any staff have contraindications with any of the pre- or post-exposure treatment options?
        - Has a suitable treatment been identified for them if they are?
- Have you documented the proficiency of the staff with the lab protocols and the associated biocontainment practices required to mitigate risks?

Invariably, your biosafety training will cover a lot of Risk Management. Risk Management includes the Biosafety Work **PRACTICES** that will help to reduce or minimize the opportunity for exposure in the lab. This includes the elimination of sharps for lab procedures by the substitution with plastic alternatives.

The identification of **PROTECTIVE EQUIPMENT** which is a combination of**:**

the personal protective clothing (lab coats or gowns, gloves – double gloves for higher risk work, face protection – chin length face shield, safety glasses or goggles and a mask); and

all engineering controls such as a biological safety cabinet, bench shields, sharps containers, vacuum system filters, biomedical waste containers, etc. that will be used to place a barrier between the biohazard and the staff.

The final element of Risk Management is to review all the **PLACES** (lab spaces) where this research will be conducted. Verify that all air flows into these laboratories and not the opposite, that all surfaces are easily cleanable, and benches are resistant to the disinfectants and other chemicals that will be used. Examine the impact of foot traffic in these locations and select times where this will be at a minimum when scheduling the times biohazards will be used there.

Once again, after you've detailed your written risk assessment and have crafted biocontainment standard operating procedures (SOP) for the proposed research or work, please contact your Biosafety Officer or assigned safety representative to schedule a walkthrough laboratory inspection to verify the suitability of the protective measures and the appropriateness of the proposed lab locations. The Biosafety Officer's report will be required by the Institutional Biosafety Committee prior to their final review and approval of your protocol to use a biohazard or regulated biological material.

Consider the 6 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	• Agent classification (See Appendix B) • Routes of infection • Infectious disease process • Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors	• Registration – See Section 2 • Biosafety Office • Biological Safety Committee • State of Connecticut - infectious agents • USDA – restricted agents • CDC – select agents • FDA/NIH - human gene therapy
Procedures	• 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.	• Written set of standard operating procedures (SOPs) with safety practices incorporated • Adherence to basic biosafety principles • Label labs, areas, and equipment housing BSL2 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	• Host immunity • 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	• 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

Table continued on next page.

Protective Equipment	• Protection (containment) for: • Aerosols – respirable size particles) <5µm • Droplets/splatter • Sharps	• Personal protective equipment (PPE): • 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	• Risk group/biosafety level requirements • Aerosol risk • Restricted access	• Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open • Labels • Containment laboratory with directional airflow

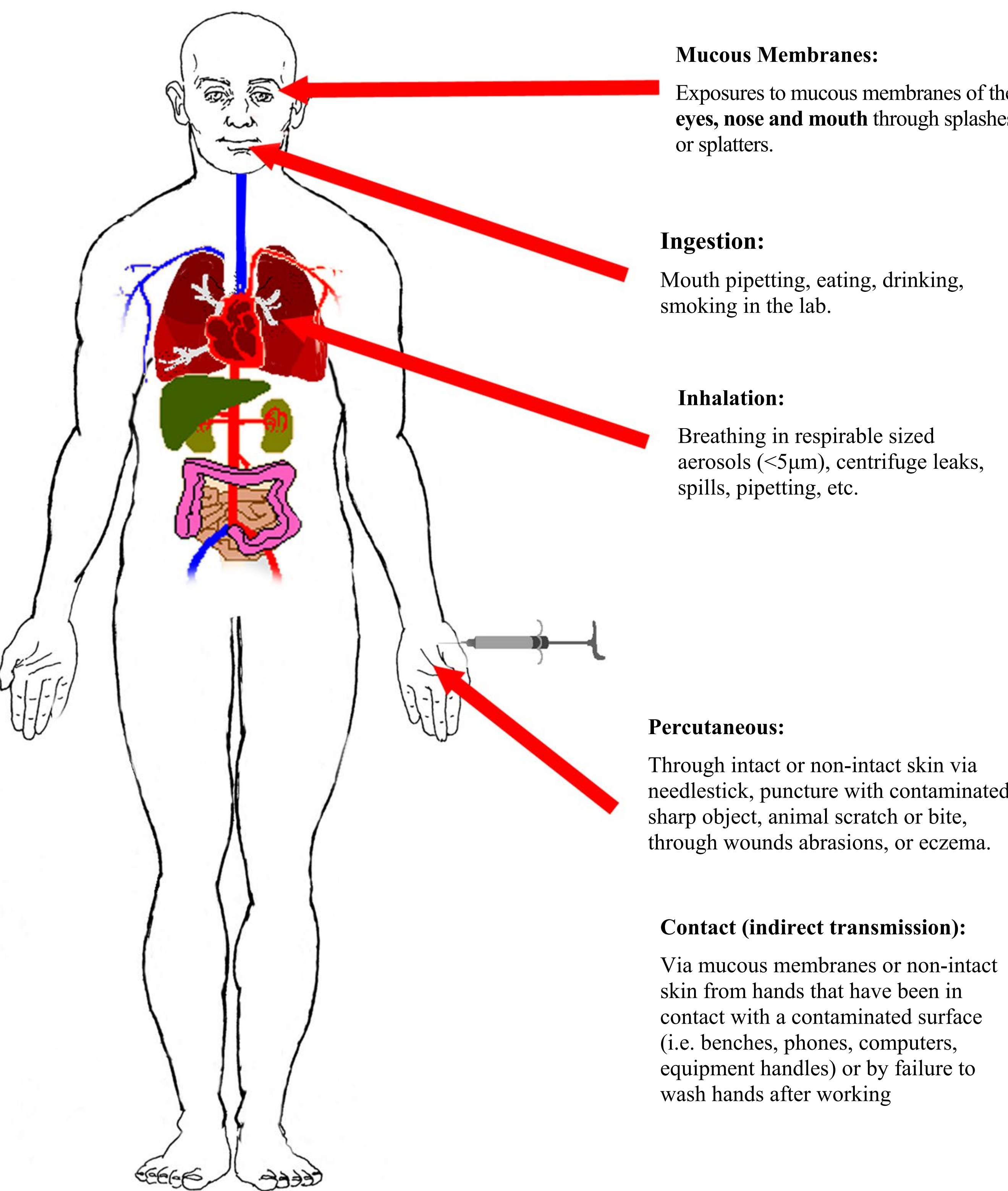
5.2 Routes of Exposures

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



Protect for the Routes of Transmission

Route of Transmission	Protection
Mucous Membranes Through mucous membranes or the eyes nose or mouth (splash, splatter).	**Face Protection:** Full-face shield or safety glasses and surgical mask, Biosafety cabinet, protective shields, good microbiological practices.
Ingestion Mouth pipetting, eating, drinking, smoking in the lab.	**Good Microbiological Practices:** Mechanical pipettors.
Inhalation Breathing in respirable sized aerosols (<5µm), centrifuge leaks, spills, pipetting, etc.	**Biosafety Cabinet:** Sealed rotors or canisters for centrifuges, safety containment equipment, HEPA filtered respirator, and good microbiological practices.
Percutaneous Through intact or non-intact skin via needlestick, puncture with a contaminated sharp object, animal scratch, bite, through wounds, abrasions, or	**Substitute plastic for glass:** Use extreme precautions with sharps, dispose immediately in rigid leakproof needle box, use animal restraints, cut resistant gloves, sleeve covers, water proof bandages, and double glove, good work practices.
Contact (indirect transmission)	**Decontamination of work surfaces and hand-washing:** Good personal hygiene (avoid touching your face with glove or non-gloved hands), do not apply cosmetics within the laboratory.

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: • 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: • 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: • 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: • 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: • 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 four 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 (BSL) 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 1–3 (BSL 1–3). 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/biosafety/publications/bmbl5/index.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: • 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: • Controlled access; • Decontamination of all waste; • Decontamination of lab clothing before laundering; • Baseline serum.	Class I or II BCSs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing; gloves; respiratory protection as needed.	BSL-2 plus: • Physical separation from access corridors; • Self-closing, double- door access; • Exhausted air not recirculated; • Negative airflow into laboratory.

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

6 Signs and Labels

Signs and labels are to be posted where biohazardous materials requiring containment at BSL2 or higher are used and/or stored. The information on the sign or label will vary depending on the use of the sign or label. The design specifications of the biohazard symbol were established in 1966 and the same specifications are still used today. The colors used on biohazard labels can vary by country; in the United States the biohazard label has an orange background with the biohazard symbol and the word "Biohazard" printed in black.

6.1 Entryway Signs

6.1.1 Door Signs

An EHS door sign must be posted at the entryway to all laboratories. Door signs provide the following information to those entering the lab including emergency responders such as police and fire personnel.

Front of door sign:

- Building and room number
- Name and phone number of all PIs using the room
- Name and phone number of research personnel knowledgeable about the materials used in the space
- Areas where biohazards requiring BSL2 or higher containment are used and/or stored will labeled with the biohazard symbol, including the word "Biohazard", and the bio safety level of the facility
- Symbols indicating that chemicals and/or radioactive materials, as appropriate, are used in the space
- Other potential hazards (e.g. gas cylinders)
- Requirements to enter the lab (e.g. safety glasses)
- The availability and location of safety equipment (e.g. an eyewash is available in the room and the hall)

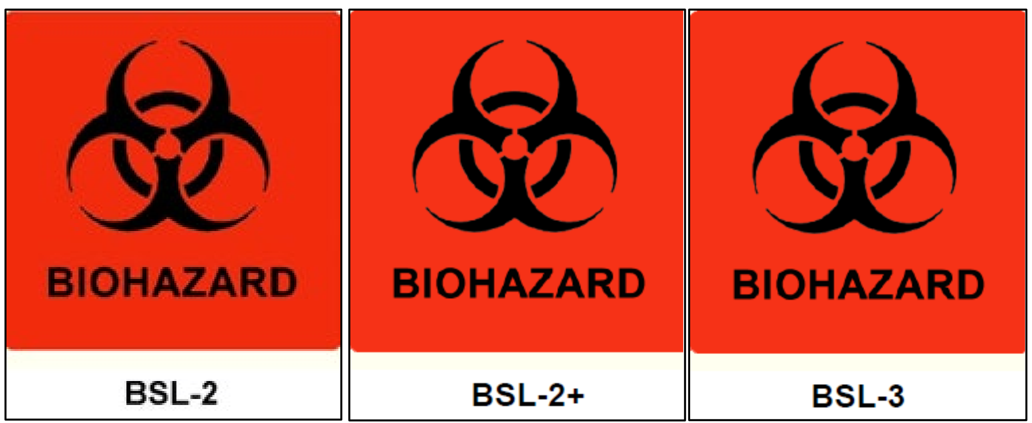
Back of door sign:

- A list of emergency contact personnel and their emergency phone number (e.g. cell phone number)
- Identification of materials requiring BSL2 or higher containment (e.g. human materials, infectious agents) used or stored in the area
- List of radionuclides used or stored in the area
- Other hazards present in the area (e.g. gas cylinders)

The Safety Advisor for your area can provide you with the required door sign.

6.1.2 Biosafety Level

Entryways to research and clinical areas where materials requiring BSL2 containment such as human blood or other potentially infectious materials must be posted with a biohazard sign that contains the universal biohazard symbol, the legend "Biohazard" and the term BSL2. Entryways to research areas that handle BL3 material must be posted with a similar sign replacing the term BSL2 with BL3.



6.2 Biohazard Door Signs

HIV and HBV research and production facilities, laboratories where work with infectious agents that cause the facility to have special entry requirements (e.g. vaccination), BSL2+ and BL3 laboratories must have a biohazard door sign posted on all access doors.

The sign includes the following:

- International biohazard symbol
- The word "Biohazard",
- The name of the infectious agent
- Any special entrance requirements
- The name and phone numbers 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. An example of a door sign is on the next page.

BIOHAZARD
BSL - 2

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY !

HAZARD IDENTITY:
Listeria monocytogenes

ENTRY REQUIREMENTS:
Pregnant women or women contemplating pregnancy who will enter this animal room should be fully informed of the potential hazards associated with Listeria monocytogenes, including potential risks to the fetus. Contact Dr. Dorothy Van Rhijn, Employee Health Physician, at 203 432 7978

THE FOLLOWING PERSONAL PROTECTIVE EQUIPMENT IS REQUIRED FOR ENTRY:

☒ GOWN ☐ SLEEVE COVERS ☒ GLOVES ☒ BOOTIES ☐ FACE SHIELD
☐ HEAD COVER ☐ RESPIRATOR PROTECTION ☒ OTHER: (as indicated on hazard SOP)

IN CASE OF EMERGENCY CONTACT:

RESPONSIBLE INVESTIGATOR: ________________________________

DAY PHONE: __________________ **EVENING PHONE:** __________________

Yale *Environmental Health & Safety*

MAIN TELEPHONE LINE: 785-3550 / EMERGENCY LINE: 785-3555
CAMPUS POLICE: 911

6.3 Labels

Inside the facility, biohazard labels shall be affixed to equipment used with or storing human blood or "other potentially infectious material"; human, animal or plant pathogens; or materials requiring a recombinant DNA registration. Containers used to transport the above materials must also be labeled with a biohazard label. The name of the material(s) requiring the lab must be identified on, or adjacent to the label as shown in the example below.

Equipment decontaminated after each use do not require a permanent biohazard label. In these situations, a biohazard label can be temporarily posted on the equipment while in use with a biohazard and the label removed once the equipment has been decontaminated.

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 Biosafety Notice (shown below) 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 and/or wiping down the exterior) and affix a Biosafety Notice to the equipment, be sure to indicate which area(s) of the equipment could not be decontaminated. Place a biohazard label on the equipment indicating the name of the biohazardous material that remains and the location of the contaminated area. The label must convey this information to all affected workers (service representatives, manufacturer, etc.). The Biosafety Notice is available on the EHS web site.

Yale *Environmental Health & Safety*

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: (Circle one)

Service/Repair Relocation Discard

Decontamination performed by: ____________________

Chemical or disinfectant: ____________________

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 Environmental Health & Safety at 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 BSL1 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 BSL2 areas.
- Keep animals not used in BSL2 experiment out of the laboratory.
- Do not smoke, eat, drink, store food or apply cosmetics in BSL2 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+ (BSL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BSL2 procedures. Generally, BL3 practices are mandated in a space designed for BSL2 work. It is preferred that the BSL2 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 BSL2+ 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 BSL2 or BSL1 use.

All manipulations of BSL2+ 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 BSL2+ are listed in Appendix C. Please consult the Biosafety Office prior to initiating any work at BSL2+.

Detailed information on BSL3 work practices is provided in the Yale Biosafety Level 3 (BSL3) manual. The BSL3 Manual is available on the Yale EHS website under Biosafety Policies and Procedures.

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.

Research materials may not be transported on any of the Yale shuttles, public transportation, or in personal vehicles. Please contact EHS if you need to transport materials between Yale campuses.

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 pre-scored 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, non-breakable 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 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 because 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.
- 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 below outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed way 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 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. If a sample is being transported between labs within buildings the sample must be placed into a rigid, leak proof secondary container with absorbent material in sufficient quantity to absorb the material in the primary container. The outside of the secondary container must be decontaminated so there is no reason to wear a glove when transporting samples. If transporting supplies or equipment between laboratories within buildings, items must be properly decontaminated prior to being brought out of the lab, there is no need to wear glove when transporting materials outside the lab. See Section 7.6: Transport of Biohazards on Campus (between labs or buildings) for more information.

The Office of Environmental Health and Safety (EHS) can provide information on gloves needed for various tasks, such as working with animals, dry ice, heat, acids, etc. Consult EHS 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
- 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 glass washing 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 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 ½ 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 EHS 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 https://ehs.yale.edu/respiratory-protection

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 BSL2 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 BSL2, 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 BSL1 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 BSL1 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 (EHS) 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 EHS 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, 1-10% bleach or other 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, 1-10% bleach 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.

EHS 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. The setup of 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.

The figure below (Figure 12) describing the proper set up of a vacuum aspiration system, including placement of traps and a filter, is copied from the CDC/NIH Biosafety in Microbiological Laboratories, 5th Edition, 2009.

Figure 12. One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

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 pad 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 pad moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pad 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 needle box. 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, biohazard bag, or box-bag unit.

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).

Thing 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 protect against 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 appropriately.
- 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 EHS. 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 viricidal 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 loose 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 less effective, as the iodine is bound to itself or the carrier molecule. 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 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 menstruum 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 tubercule 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. Cpds	0.1-2.0%	+	+			+					+	+	+	+		+	+	+	+	+	
Phenolic Cpds	1.0-5.0%	+	+	b		+	+			+			+	+		+	+	+	+	+	
Chlorine Cpds	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 section 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 ($\geq$ 1 liter) of Biosafety Level 1 material. The following items should be included in the spill kit:

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

Household bleach is recommended as a standard disinfectant in this guide. Other disinfectants may be used provided the disinfectant is effective against the agents in use at the appropriate dilutions and contact time. For spills involving human materials, including human cell lines, use bleach or a disinfectant registered with the Environmental Protection Agency as tuberculocidal to comply with the Occupational Health and Safety Administration Bloodborne Pathogens Standard.

If you have any questions regarding biological spill response procedures or decontamination, representatives from the EHS Occupational Health and Safety section are available by calling 785 - 3550. All spills in a BL3 laboratory shall be reported to EHS immediately.

11.2 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 Yale Health, Acute Care (432-0123). Medical Area employees may also go to the Yale-New Haven Hospital Emergency Room (688-2222).

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

11.3 Biosafety Level 1 (BSL1) Spill

Notify others in the area, to prevent contamination of additional personnel and environment.
Remove any contaminated PPE and clothing and wash exposed skin with soap and water.

Clean-up of BL1 Spill

- Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill working from the edge of the spill towards the center. Allow suitable contact time, at least 15 min.
- 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 bag.
- Wash hands with soap and water.

11.4 Biosafety Level 2 (BSL2) Spill

- Avoid inhaling aerosols and leave the room; notify others to leave as well. Close door, post a sign on the laboratory door indicating there is a biohazard spill, do not enter, and the time of the spill.
- Remove contaminated PPE, 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, call the Biosafety Office at 785-3550.

Reporting of Spills Involving rDNA Materials

Spills or accidents in BSL2 laboratories involving recombinant DNA materials resulting in an overt exposure must be immediately reported to EHS, who will assist in reporting the incident to the NIH Office of Biotechnology Activities (OBA) as required under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines).

Clean-up of BSL2 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 PPE (lab coat, face protection which can consist of a full-face shield or surgical mask and safety glasses, gloves, 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. Only personnel that have completed Respirator Protection Training and are medically cleared may wear HEPA respirators.
- Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill working from the edge of the spill towards the center. 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 the paper towels and any sharps with forceps or tongs and discard into the appropriate biomedical waste container. Always use mechanical means to pick up the spill cleanup material since there may be sharps under the paper towels. The paper towels are placed over the sharps to decontaminate the sharps prior to removal. 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.
- Spray the area with 10% household bleach solution and allow to air dry (or wipe down with disinfectant-soaked towels after a 15-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 soap and water.

11.5 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 PPE into a biohazard bag.
- Wash your hands with soap and water.

11.6 Spill in a Biological Safety Cabinet

- Leave the biological safety cabinet blower on and begin cleanup immediately.
- While wearing PPE (gloves and lab coat or gown) cover the spill area with paper towels or disinfectant soaked paper towels. Do not place your head into the biosafety cabinet to clean the spill, keep your face behind the viewscreen.
- If necessary, apply disinfectant to the drain pans and catch basins below the work surface. Be sure the drain valve is closed before disinfecting the area under the work surface.
- Wipe cabinet walls, work surfaces, and inside the viewscreen with disinfectant.
- Lift the front exhaust grill and work surface; wipe all surfaces with disinfectant. Be sure no paper towels or soiled debris are drawn into the area under the spill tray
- If the drain pans and catch basins under the work surface have had disinfectant applied to them, absorb the disinfectant using paper towels or lab matting. If necessary, place a container under the drain valve and drain the disinfectant under the work surface into the container. Wipe the areas under the work surface to remove residual disinfectant.
- If bleach was used, follow by wiping with 70% ethanol to remove any corrosive residues.
- Wash hands and exposed skin with soap and water.
- Autoclave all cleanup materials and disposable PPE.
- Notify your PI or supervisor.

If the blower/motor of the biosafety cabinet is below the work surface and the spill overflows the drain pan/catch basin under the work surface notify Occupational Health and Safety. A more extensive decontamination of the biological safety cabinet may be required.

11.7 Centrifuge Spill

- Always use sealed safety buckets or sealed rotors with O-rings.
- Examine O-ring and replace if worn, cracking or missing. Check tubes and bottles for cracks and deformities before each use.
- Wait five minutes before opening the centrifuge following the end of a run with potentially hazardous biological material. If a spill is identified after the centrifuge lid is opened, carefully close the lid, evacuate the laboratory, and close the laboratory door. Remain out of the laboratory

for at least 30 minutes. Post a sign on the laboratory door indicating there is a biohazard spill, do not enter, and the time of the spill.

- Remove any contaminated PPE and place into a biohazard bag. Wash hands and any exposed skin surfaces with soap and water.
- Notify your supervisor and the Biosafety Office (785-3550).

After 30 minutes

- Enter the lab with PPE and spill cleanup materials. Full-face protection, lab coat, gloves and utility gloves should be worn.
- Transfer rotors or buckets to a biological safety cabinet. Immerse rotor/buckets in 70% ethanol or a non-corrosive disinfectant effective against the agent in use and compatible with the material the rotor is made of. Allow at least a one-hour contact time. Intact tubes may be wiped down and placed into a new container. Handle any broken glass with forceps and discard into a sharps container.
- Carefully retrieve any broken glass from inside the centrifuge using forceps and discard into a sharps container. Smaller pieces of glass may be collected with cotton or paper towels held with forceps. Carefully wipe the inside of the centrifuge with disinfectant. Spray the inside of the centrifuge with disinfectant and allow to air dry. If bleach was used, follow by wiping with 70% ethanol to remove any corrosive residues.
- Place contaminated items and disposable PPE in an autoclave bag and autoclave. Decontaminate reusable PPE
- Wash hands with soap and water.

11.8 Spill of a Biohazardous Radioactive Material

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

Follow the steps below as soon as the spill happens:

- Avoid inhaling aerosols while leaving the room; and notify others to leave as well.
- Close door(s) to the area and post a warning sign indicating there is a spill, do not enter, and the time of the spill.
- Be aware that you may be spreading contamination, both biological and radioactive, so reduce movement once in a safe area.
- 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.
- If material splashed onto anyone, monitor the person for radioactive contamination using the appropriate radiation detection instrument.
- Wash all exposed skin with soap and water for 15 minutes.
- Call the EHS Emergency number at (203) 785-3555 to report the spill.
- Inform supervisor of the spill.
- Monitor all personnel that were in the vicinity of the spill for radiation. If personal radioactive contamination is found, record the counts per minute found.

Clean-Up of a Biohazardous Radioactive Material

Before any clean up, consider the type of radionuclide, characteristics of the microorganism, and the volume of the spill. This information is important when determining which disinfectant to use on the spill. For isotope clean-up procedures contact the EHS at (203) 785-3550.

- 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 Radiation Safety that it is safe to enter the lab.
- Put on PPE (gown, face protection, which can consist of a full-face shield or surgical mask and safety glasses, double gloves, and shoe covers). Depending on the nature of the biological material involved, it may be advisable to wear a HEPA-filtered respirator instead of a surgical mask.
- **Note:** Everyone wearing a respirator has to complete respirator training, medical clearance, and be fit tested to the respirator. If these requirements have not been completed a respirator cannot be worn.
- Cover the area with disinfectant-soaked towels, and carefully pour disinfectant around the spill working from the edge of the spill towards the center. 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 radioactive iodine containing material, radioiodine gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.
- Handle any sharp objects with forceps and discard into a sharps container. The container has to be labelled with both a biohazard label and a radioactive materials label if the items have not been biologically decontaminated.
- Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- Pick up the paper towels used to cover the spill using forceps or tongs and place the biologically decontaminated waste, along with all PPE contaminated with radioactive materials, into an approved radioactive waste container. Label the container according to Radiation Safety Guidelines. Contaminated PPE must also be biologically decontaminated prior to disposal as radioactive waste.
- **Do Not** autoclave the waste unless this action is approved by the Radiation Safety Officer. If waste cannot be autoclaved, please contact the EHS Biosafety and Radiation Safety offices for an alternative decontamination method (to be determined based on the waste profile).
- Wash hands and exposed skin areas with soap and water; 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 radioactive contamination, determine if it is fixed or removable and handle accordingly.
- Discard items contaminated with radioactive materials into the appropriate radioactive waste container:
    - Place the contaminated item(s) on absorbent paper.
    - Spray disinfectant (10% household bleach) on the contaminated areas and allow a 20-minute contact time.
    - Wrap the item(s) inside the paper and dispose of as radioactive waste.
- Wash hands with soap and water.

11.9 BSL3 Spill Response Procedures

Use the following guidelines for response to spills of BSL3 material outside of the biological safety cabinet or any other incident that may have generated an aerosol in the containment laboratory such as failure of physical containment devices during centrifugation.

Immediate Action

- Hold breath and leave room immediately. Notify others in the room to evacuate immediately.
- Remove personal protective equipment (PPE) in the airlock or access zone; turn potentially contaminated clothing inward; remove gloves last and wash any exposed skin areas with antiseptic soap and water.
- Post a Biohazard Spill Sign on the BL3 lab door.
- Notify Principal Investigator (PI) and Environmental Health and Safety (EHS).
- Do not reenter laboratory until it has been cleared for reentry by the PI or EHS. In general, a period of at least 30 minutes should be allowed before cleanup is attempted, but the time is contingent upon the supply and exhaust features of the lab.

Cleaning the BSL3 Spill

If the spill involves radioactive material also contact Radiation Safety.

Once clearance has been given to reenter the spill area by the PI, in consultation with EHS, don the appropriate PPE and enter the spill area.

- While wearing PPE cover the spill area with paper towels or disinfectant soaked paper towels.
- Slowly pour concentrated disinfectant around the edge of the spill working towards the center (this will avoid enlarging the spill area). **Avoid splashing and the creation of aerosols during this step.**
- Allow a 15-20-minute contact time for the disinfectant.
- While waiting, decontaminate the surrounding floor and work surfaces where splashes or larger aerosols may have settled around the spill. Use disinfectant soaked paper towels to wipe these areas (1-10% household bleach solution is suitable for this purpose).
- After the 15-20 minute contact time place the soiled paper towels into a biohazard bag.
- Repeat the decontamination procedure.

After performing the decontamination procedure:

- Decontaminate any reusable items, such as forceps, by wiping debris off the items then soaking in a disinfectant solution. A soak in 1-10% household bleach solution for 15-20 minutes is sufficient. Since bleach is corrosive, follow the decontamination with a 70% ethanol wipe to remove any corrosive residues.
- Remove PPE, turning any exposed areas inward, and place into a biohazard bag. Generally, gloves are removed last, however, to avoid touching the face with gloved hands, remove gloves just before removing masks or eyewear.
- Decontaminate the exterior of biomedical waste containers.
- Wash hands well with soap and water for at least 15-30 seconds.
- Autoclave all waste generated from the spill cleanup. Use fresh gloves for transport to the autoclave and wash hands after removing gloves.

12 Select Agents

Select agents are materials that have been identified by the U.S. Government as agents with potential for 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 maintains 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 below, and can also be accessed from the web sites below:

The Select Agent Registry home page: http://www.selectagents.gov/ (or search the internet for the term Federal Select Agent Program.

Additional web links for select agent information are provided below.

- CDC Division of Select Agents and Toxins: https://www.cdc.gov/phpr/dsat.htm
- USDA Agriculture Select Agent Services: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/SA_Ag_Select_Agent

The federal select agent regulations were updated and changes have taken effect as of March 21, 2017. The most significant changes are an update of the select agents and toxins list (as reflected in Appendix A of this plan) and the change in the permissible amounts of toxins allowed per laboratory for exemption from registration. The list of Tier 1 select agents and toxins has also been updated and are also included in Appendix A. Institutions that possess or work with Tier 1 agents will have to implement additional personnel screening and ongoing personal screening requirements beyond the current federal background checks. Yale University is not currently registered for Tier 1 agents. A personnel screening program will be developed prior to the receipt of Tier 1 agents. In addition to the enhanced screening requirements for personnel with access to Tier 1 select agents, additional physical security measures have been added for locations where Tier 1 select agents are either stored or used. Yale University's current select agent laboratory is in conformity with the additional physical security measures that are required for research with Tier 1 select agents and toxins.

National concerns over select agents have led to an expansion in security requirements for these materials. As a result, entities and researchers in possession of these materials have additional obligations and responsibilities for their safe storage, use, transfer, and disposal.

12.1 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 (EHS).
- Register with the State of Connecticut Department of Public Health through Yale EHS. 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.

12.2 List of Select Agents and Regulated Toxins

The list of Select Agents below is current as of September 13, 2019. Please refer to the Federal Select Agent Program select agent and toxin list at: https://www.selectagents.gov/selectagentsandtoxinslist.html.

Select Agent Regulations may be found at: https://www.selectagents.gov/regulations.html

The list of excluded agents and toxins can be found at: https://www.selectagents.gov/SelectAgentsandToxinsExclusions.html

HHS and USDA Select Agents and Toxins
7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS Select Agents and Toxins

1. Abrin
2. *Bacillus cereus* Biovar *anthracis**
3. Botulinum neurotoxins*
4. Botulinum neurotoxin producing species of *Clostridium**
5. Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
6. *Coxiella burnetii*
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol
9. Eastern Equine Encephalitis virus
10. Ebola virus*
11. *Francisella tularensis**
12. Lassa fever virus
13. Lujo virus
14. Marburg virus*
15. Monkeypox virus
16. Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
17. Ricin
18. *Rickettsia prowazekii*
19. SARS-associated coronavirus (SARS-CoV)
20. Saxitoxin

South American Haemorrhagic Fever viruses:

21. Chapare
22. Guanarito
23. Junin
24. Machupo
25. Sabia

26. Staphylococcal enterotoxins (subtypes A, B, C, D, E)
27. T-2 toxin
28. Tetrodotoxin

Tick-borne encephalitis complex (flavi) viruses:

29. Far Eastern subtype
30. Siberian subtype

31. Kyasanur Forest disease virus
32. Omsk hemorrhagic fever viru
33. Variola major virus (Smallpox virus)*
34. Variola minor virus (Alastrim)*
35. *Yersinia pestis**

Overlap Select Agents and Toxins

36. *Bacillus anthracis**
37. *Bacillus anthracis* Pasteur strain
38. *Brucella abortus*
39. *Brucella melitensis*
40. *Brucella suis*
41. *Burkholderia mallei**
42. *Burkholderia pseudomallei**
43. Hendra virus
44. Nipah virus
45. Rift Valley fever virus
46. Venezuelan equine encephalitis virus

USDA Select Agents and Toxins

47. African horse sickness virus
48. African swine fever virus
49. Avian influenza virus
50. Classical swine fever virus
51. Foot-and-mouth disease virus*
52. Goat pox virus
53. Lumpy skin disease virus
54. *Mycoplasma capricolum*
55. *Mycoplasma mycoides*
56. Newcastle disease virus
57. Peste des petits ruminants virus
58. Rinderpest virus*
59. Sheep pox virus
60. Swine vesicular disease virus

USDA Plant Protection and Quarantine (PPQ) Select Agents and Toxins

61. *Coniothyrium glycines* (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)
62. *Peronosclerospora philippinensis (Peronosclerospora sacchari)*
63. *Ralstonia solanacearum*
64. *Rathayibacter toxicus*
65. *Sclerophthora rayssiae*
66. *Synchytrium endobioticum*
67. *Xanthomonas oryzae*

*Denotes Tier 1 Agent

12.3 Tier 1 Select Agents

A subset of select agents and toxins have been designated as Tier 1 because these biological agents and toxins present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety:

Tier 1 Select Agents and Toxins		
HHS Agents and Toxins	**Overlap Agents**	**USDA Agents**
• *Bacillus cereus* Biovar *anthracis* • Botulinum neurotoxins • Botulinum neurotoxin producing species of *Clostridium* • Ebola virus • *Francisella tularensis* • Marburg virus • Variola major virus (Smallpox virus) • Variola minor virus (Alastrim) • *Yersinia pestis*	• *Bacillus anthracis* • *Burkholderia mallei* • *Burkholderia pseudomallei*	• Foot-and-Mouth Disease virus • Rinderpest virus

Entities that possess, use, or transfer Tier 1 select agents and toxins must adhere to the additional personnel screening and ongoing personal screening requirements. Additional physical requirements are also required for Tier 1 Select Agents.

The Yale Select Agent laboratory has been designed to meet the additional physical security requirements. However, the finalized program for personnel screening has not been set forth as of this version of the manual as Yale University is not currently registered for experiments involving Select Agents.

12.4 Permissible Toxin Amounts

The following toxins are not regulated if the amount under the control of a Principal Investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the table below.

HHS Toxins [§73.3(d)(3)]	*Amount*
Abrin	1,000 mg
Botulinum neurotoxins	1.0 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Note: Toxin quantities shown are maximum quantities of toxins, in aggregate, per PI, allowed by USDA/CDC. Note: Yale's internal watch list limits PIs to only 50% of the quantities shown here.

12.4.1 Toxin due Diligence Requirements

All Yale Principal Investigators in possession of ANY QUANTITY of the 9 Select Agent Toxins listed above must retain a record of ALL transfers of ANY QUANTITY of these toxins outside their laboratory. The required documentation must include the following information:

- Name of the recipient
- Toxin and quantity transferred
- Purpose of use (knowledge of recipient's legitimate need for the toxins)

12.4.2 Reporting Suspected Violations or Suspicious Activity

If a Yale Principal Investigator in possession of ANY QUANTITY of the 9 Select Agent Toxins listed above detects suspicious activity associated with a request for toxin or suspicious activity associated with a shipped toxin, s/he must immediately notify the Yale University EHS Contact or the Alternate EHS Contact and the Federal Select Agent program at the contact information below:

Yale's Contact: Mr. Kevin Charbonneau: 203-737-2139 (office) 203-410-8527 (cell)
Yale's Alternate Contact: Mr. Ben Fontes: 203-737-5009 (office) 203-410-6223 (cell)

Email CDC: LRSAT@cdc.gov
CDC Select Agent Office: 404-718-2000

12.5 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 EHS or contact your EHS 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 EHS of your intent to possess, use or transfer Select Agents.
- Complete an update of your Biological General 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 FBI Form FD961 and file with Yale EHS for registration with applicable federal entity
- Complete 2 sets of FBI fingerprint cards for initiation of background investigation check
- Complete all Yale EHS applicable training programs (Biosafety, Bloodborne Pathogens, Laboratory Safety, Biosafety Level 3, Select Agent Biosecurity, and Shipping/Transport of Hazardous Biological Agents).
- Complete a Yale EHS Request to Use Infectious Agents Form(s) and Researcher Experience Form.
- Complete the Yale EHS researcher experience requirements (for BSL2+ or BL3 agents)
- Satisfactory completion of EHS 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 EHS, 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). **This is only a general description of the process and it could take 6 to 9 months (or longer) from the time of the initial application to the time a federal inspection is scheduled for the proposed select agent research.**

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.

12.6 Discovery of Select Agents or Unknown Samples

Please notify EHS 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.

12.7 Intrafacility Transfer of Select Agents

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

12.8 Destruction of Select Agents or Unknown Samples

Select Agent pathogens or toxins may not be destroyed until Yale EHS and the applicable government institution has provided approval for the destruction. Once approval has been granted for the destruction of Select Agents, Yale EHS 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 EHS Biosafety Office or your EHS Safety Advisor at 785-3550.

12.9 Additional Information

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

Yale EHS: https://ehs.yale.edu/select-agents-bsl-three

Centers for Disease Control and Prevention: http://www.selectagents.gov/

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

United States Department of Agriculture:

https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/SA_Ag_Select_Agent

13 Research Involving Recombinant and Synthetic Nucleic Acid Molecules

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines) are a set of research requirements for any institutions receiving funding from the NIH for molecular biology research. As Yale receives NIH funding for this type of research, the NIH Guidelines must be followed by every PI at the University, regardless of their source of funding for this research, which is a central condition of the NIH funding agreement.

13.1 Principal Investigator Responsibilities Under the NIH Guidelines

Principal Investigators are responsible for full compliance with the NIH Guidelines. Core responsibilities include:

- Identifying if their research is subject to the NIH Guidelines
- Determining which section of the Guidelines are applicable to their work
- Proposing an appropriate biocontainment level (BSL) to ensure that the research is performed safely
- Obtaining authorization from the Yale Biological Safety Committee prior to starting any research that is subject to the NIH Guidelines

Non-exempt recombinant DNA experiments, which must be registered with, and approved by, the Biosafety Committee prior to initiation, are defined in the left-hand column of the Registration and Approval of rDNA Experiments document found in appendix E of the Biosafety Manual. These include experiments that involve:

- The deliberately transfer a drug resistance trait to a microorganism
- Human gene transfer
- Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of <100 μg/kg body weight
- Human or animal pathogens as host-vector systems
- Cloning of DNA or RNA from certain pathogens
- Recombinant DNA work in whole animals or plants
- Large-scale DNA work with more than 10 liters of culture

More specific information on the classification of non-exempt recombinant DNA experiments is provided below. Please review the examples of non-exempt experiments and ensure that your laboratory is registered for proposed rDNA experiments prior to initiation where applicable. The examples provided include work that is commonly miss-classified by investigators.

The end of this Section of the Biosafety Manual includes relevant fact sheets from the NIH Office of Biotechnology Activities that are useful resources for Principal Investigators. The following table from the NIH provides an overview of rDNA experiments and the level of review required for each category.

13.1.1 Level of Review for rDNA Experiments

Level of Review	Example of rDNA Experiment	NIH Guideline Section
IBC and NIH Director Approval	Experiments that compromise the control of disease agents in medicine through deliberate transfer or a drug resistance trait	III-A
IBC Approval and NIH review	Deliberate formation of rDNA containing genes coding for a toxin with an LD50 < 100 ng/kg	III-B
IBC and IRB and FDA Approval	Introduction of rDNA into human subjects (Human gene transfer)	III-C
IBC Approval before initiation	Wide range (rDNA experiments involving pathogens, defective vectors, animals, plants, and large scale projects)	III-D
IBC Notice at initiation	Creating transgenic rodents, low risk rDNA Plant experiments	III-E
Exempt – registration not required	Those do not represent a significant risk to health or the environment	III-F

Principal Investigators are responsible for ensuring that their researchers are trained in safe work practices and that everyone working in the laboratory is aware of the emergency response procedures that must be followed. Staff must also be alerted of the rationale for any special medical surveillance restrictions or immunizations. Once work has been initiated, Principal Investigators must also supervise and monitor staff for their adherence to safety protocols.

If the scope of research changes significantly, the Principal Investigator must update their rDNA protocol with the Yale Biological Safety Committee and await confirmation in writing that the protocol change has been approved.

It is also important that Principal Investigators report any significant problem, such as a violation of the NIH Guidelines or any significant research related accidents, exposures or illnesses to the Yale Biological Safety Committee and the Environmental Health and Safety Office (both can be reached at 203-785-3550).

13.2 Overview of Recombinant DNA Experiments Covered by The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) April 2019

The NIH Guidelines describe experiments that must be registered and approved by Institution's Biological Safety Committee and those experiments that are exempt from the Guidelines and do not require registration. For institutions that receive funding from the NIH for molecular biology research, the Guidelines become a condition of the receipt of funding. For Yale to continue to receive funding from the NIH, ALL researchers MUST COMPLY with the NIH Guidelines, regardless of funding source for molecular biology research. Persistent failure of an institution to comply with the NIH Guidelines can lead to suspension of research privileges. Systemic failure to comply with the Guidelines may result in the freezing of funds directed at an institution by the NIH.

The phrase rDNA is used throughout this document and refers to recombinant or synthetic nucleic acid molecules to reflect earlier changes in the March 2013 and November 2013 versions of the NIH Guidelines.

Link to current NIH Guidelines: https://osp.od.nih.gov/biotechnology/nih-guidelines/

13.2.1 Categories of rDNA Work That Require Registration

1. Cloning a therapeutic antibiotic resistance gene into a human, animal or plant pathogen, if the transfer could compromise the ability to treat or control the disease. (Section III-A-1)

Note: Registration with the institution's Biological Safety Committee is still required even if:

- this drug resistance is acquired naturally (the Committee will determine if it is a Major Action or not and communicate with the NIH Office of Science Policy (OSP) as needed);
- the transferred resistance gene is related to a drug that is an end of the line alternative treatment (2nd, 3rd, 4th, or 5th line drug) - the Committee will determine if the proposed research qualifies as a Major Action or not;
- the drug was used years ago, but is not the preferred treatment today (it may be the only treatment in developing countries);
- the drug is only used to treatment a very small portion of the population (i.e. those with specific contraindications to front line drugs)
- Working with antibiotic resistance strains of pathogens also require registration (even if you did not create them) – please submit a registration form to the Biological Safety Committee

Examples:

- Cloning a gene for Erythromycin resistance into Borrelia burgdorferi (may not qualify as a Major Action, but the Institutional Biosafety Committee must review prior to initiation)
- Cloning a gene for Chloramphenicol resistance into *Rickettsia typhi*
- Cloning a gene for Pyrimethamine resistance into *Toxoplasma gondii*
- Cloning a gene for Rifampin resistance into *Mycobacterium tuberculosis*

Caution:

- Be careful when using old plasmids for cloning experiments involving pathogens. Many of the old plasmids carry genes for antibiotics that have been used therapeutically or are related to front line drugs.
    - Avoid using these plasmids when working with related pathogens;
    - Verify that the antibiotic resistance gene is not in a location on the plasmid that can be transferred to the pathogen via a double cross over event.

Website to visit for additional information:

NIH OSP FAQ – Major Actions https://osp.od.nih.gov/biotechnology/nih-guidelines-faqs/

2. Cloning DNA encoding for a low LD50 toxin or work with vectors that express toxins with a low LD50 (< 100 µg/kg body weight). (Section III-B-1)

Examples of toxins with low LD50's are:

- Botulinum toxin
- Staphylococcal enterotoxin B
- Tetrodotoxin
- *Clostridium tetanus* toxin

Website to visit for additional information:

Univ. of Florida – Toxin Lists: http://www.ehs.ufl.edu/Bio/toxin.htm

3. Human Gene Transfer Experiments (Section III-C-1)

The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the NIH Guidelines.

This includes the transfer of DNA with defective viral vectors, such as retroviral, adenoviral and lentiviral vectors, along with the use of liposomes and other methods of delivery.

Human gene transfer experiments with synthetic nucleic acid molecules also require registration if any of the following criteria are met: The synthetic nucleic acid molecules:

- Contain more than 100 nucleotides; or
- Possess biological properties that enable integration into the genome (e.g. cis elements involved integration); or
- Have the potential to replicate in a cell; or
- Can be translated or transcribed

These experiments also require approval from the institutional review board at the institution (Yale Human Investigation Committee) and the U.S. Food and Drug Administration.

Websites to visit for additional information:

Yale HGT experiments: https://ehs.yale.edu/human-gene-transfer

NIH OSP Points to Consider for IBCs Reviewing Human Gene Transfer Protocols:

https://osp.od.nih.gov/biotechnology/nih-guidelines-faqs/

4. ***rDNA Experiments involving the use of a human, animal or plant pathogen (whether the recombinant or synthetic nucleic acid molecules originated from your lab or another). (Section III-D-1, III-D-2, III-D-3)***

Examples:

- Cloning a gene into a pathogen (i.e. expressing a gene into VSV, Vaccinia Virus, Tobacco Mosaic Virus, Mouse Cytomegalovirus)
- Cloning a pathogen into a lower eukaryotic or prokaryotic cell;
- Using a defective pathogen vector with or without helper virus in cell culture or animal experiments, examples include:
  - Poxviruses (Vaccinia)
  - Herpesvirus vectors (HSV)
  - Lentivirus vectors (HIV, FIV based)
  - Retroviruses (murine retroviruses)
  - Adenoviruses
  - Adeno-Associated Virus vectors
  - Vesicular Stomatitis Virus vectors
  - Sindbis Virus vectors

Tools and resources can also be found on Yale EHS's website for rDNA research:

https://ehs.yale.edu/recombinant-dna

A helpful guidance document developed by Stanford University for the classification of experiments involving defective viral vectors can be accessed at the following website:

https://ehs.stanford.edu/topic/biosafety-biosecurity/viral-vectors

Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.

5. ***Cloning DNA or RNA from Risk Group 3 or Risk Group 4 human pathogens, restricted animal or plant pathogens, or Select Agents. (Section III-D-2)***

Any rDNA experiments with these materials must be registered with and approved by the Yale IBC, even if you are working with only short segments of nucleic acid molecules from these agents.

Websites to visit for additional information:

Please refer to Appendix B: Classification of Etiologic Agents on the Basis of Hazard for the Risk Groups assigned to the pathogen you intend to work with or access the NIH Guidelines on the NIH Office of Science and Policy web site at:

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

American Biological Safety Association Risk Group Classifications of Etiologic Agents:

https://absa.org/

https://my.absa.org/tiki-index.php?page=Riskgroups

Pathogen Safety Data Sheets from Health Canada are also available for some human pathogens and can be accessed here:

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

List of "Veterinary Pathogens of Significance" (can be found in Appendix D of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, 2009)

https://www.cdc.gov/labs/BMBL.html

Select Agent List:

http://www.cdc.gov/od/sap/docs/salist.pdf

6. rDNA Experiments involving whole animals, plants, and arthropods (and insects) Section III-D-4, III-D-5, III-E-3

Experiments in this category include:

- Experiments involving toxins, pathogens, defective vectors, and other genetically modified materials used in animal, plants or insects.
- Creation of transgenic animals:
    - i. Mice, rats
    - ii. Zebrafish
    - iii. Drosophila, butterflies
    - *iv. Caenorhabditis elegans*
    - v. Other

Note: For rodents only, the purchase or transfer of transgenic rodents is exempt from the NIH rDNA Guidelines and does not require registration (if the transgene used does not code for a toxic, virulent or oncogenic sequence).

Purchase is defined as buying a transgenic rodent that has been created by another entity outside of your laboratory.

The transfer of a transgenic rodent to your laboratory is also exempt (provided the transgene doesn't code for toxic, oncogenic or potentially harmful gene).

Transfer is defined as the acquisition into your research lab of a transgenic animal created (made) by another entity.

Note: In each case above, you may have designed or created the gene that has been inserted into the developing embryo of the transgenic rodent, but if you are not the group that has performed the actual procedure (i.e. the lab that inserted the gene into the embryo), you are exempt from the rDNA Guidelines. **If your lab will insert the gene into the embryo, you must register this work.**

Knock-out Animals

Knock-out (gene silencing, gene ablation, etc.) rodents are exempt from the NIH Guidelines as long as the method to generate the knock-out animal does not leave any "new" genetic material behind in the genome after the procedure. If DNA from the molecule used to create the knock-out is permanently inserted into the genome, the experiment will require registration with the Yale Biological Safety Committee.

Exemption for Breeding Transgenic Rodents

Note: Generation of transgenic **rodents** by breeding to create a new strain shall be EXEMPT from the NIH Guidelines if the following criteria are met.

- Both parental rodents can be housed under BSL1 containment; AND
- Neither parental transgenic rodent contains the following genetic modifications:
  - Incorporation of more than 50% of the genome of an exogenous eukaryotic virus from a single family of viruses; OR
  - Incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); AND
- The transgenic rodent that results from this breeding is not expected to contain more than 50% of an exogenous viral genome from a single family of viruses.

This exemption DOES NOT pertain to other transgenic animals such as zebrafish, drosophila, rabbits, pigs, etc. It also DOES NOT pertain to transgenic experiments involving plants.

7. Large Scale rDNA Experiments (Section III-D-6)

Any rDNA experiments at any level or Risk Group, including exempt and non-exempt experiments that generate a volume of culture that is in excess of 10 liters require registration with the Yale Biological Safety Committee.

Examples include: Growing 10.1 L of *Saccharomyces cerevisiae* in a fermentation apparatus to get a sufficient yield of the desired protein.

Growing up five 2 L flasks of E. coli K-12 cultures expressing your gene of interest would not qualify as a large scale experiment under the NIH Guidelines, but given the high volume, please register this volume of work with the Institutional Biological Safety Committee in order for an appropriate risk assessment of the proposed work.

8. Synthetic Nucleic Acid Experiments that are covered by the Guidelines:

- Research that presents biosafety risks equivalent to rDNA research that is subject to the NIH Guidelines such as research with a genetically modified virus or a vector derived solely by synthetic techniques.
  - The molecules can replicate
  - They can generate nucleic acids that can replicate in a living cell
  - They can integrate into a host cell's DNA
  - They produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms/kilogram body weight
  - They synthesize an organism that doesn't occur naturally outside of a laboratory setting (i.e. 1918 H1N1 Influenza)

13.2.2 Synthetic Nucleic Acid Experiments that are EXEMPT from the Guidelines

- Introduction of certain synthetic nucleic acids into a biological system that is not expected to present a biosafety risk that requires review by the IBC
- Introduction of synthetic nucleic acid molecules into biological systems akin to processes of nucleic acid transfer that already occur in nature.
- Experiments with synthetic nucleic acid molecules that are not contained in cells, organisms or viruses
- Those synthetic nucleic acid molecules that meet the following criteria shall be exempt:

1) Those that can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g. oligonucleotides or other synthetic that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and
2) Those that are not designed to integrate into DNA, and
3) Those that do not produce a toxin that is lethal for vertebrates at and LD50 of less than 100 nanograms per kilogram body weight.

An example of an exempt synthetic nucleic acid molecule is a synthetic short interfering RNA (siRNA) that targets an HIV viral protein required for transcription activation, even if this siRNA is injected into animals or used in cell culture.

- Also exempt are those synthetic nucleic acid molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists outside of a laboratory setting. (Research with nucleic acid sequences for organisms that do not currently exist in nature outside of the laboratory setting would not be exempt (e.g. an identical copy of the 1918 H1N1 influenza virus)
- The chemical synthesis of nucleic acids (the NIH Guidelines only apply once synthetic nucleic acids are placed in a biological system).

13.3 Yale Biological Safety Committee Meeting rDNA Protocol Review

Yale University
Environmental Health & Safety

135 College Street, 1st Floor
New Haven, Connecticut 06510
Telephone: 203 785-3550
www.yale.edu/oehs
Fax: 203 785-7588

Yale Biological Safety Committee (BSC) Meeting rDNA Protocol Review

Principal Investigator: **Benjamin Fontes**
Yale BSC rDNA Protocol #: **#13-00**

The Yale Biological Safety Committee Registration # assigned to your protocol

Title of rDNA Protocol: Use of Viral Vectors for Transfection Experiments

Section 1: Committee Action

☐ The Yale Biological Safety Committee has reviewed your rDNA application and has found it to be in compliance with the NIH Guidelines. **The Protocol was approved as follows:**

This Box will be checked if your protocol has been approved

This section informs you of the biocontainment level that has been assigned to your protocol as well as the Section from the NIH Guidelines that best fit your experiments

For Cell Culture Experiments:	For Animal Experiments:
☐ BSL-1 Exempt	☐ ABSL-1 Exempt
☐ BSL- 1	☐ ABSL-1
☐ BSL- 2	☐ ABSL-2
☐ BSL-2 Enhanced	☐ ABSL-2 Enhanced
☐ BSL-3	☐ ABSL-3
☐ BSL-2 w/ Universal Precautions for work with human material	
NIH Classification: III-D-1-a, III-D-3-b	NIH Classification: III-D-1-a. III-D-4-b

Please see Section 2 for specific protocol requirements and Section 3 for general rDNA protocol requirements.

Your approval and renewal dates for your protocol

Protocol Approval Date: March 21, 2013 Protocol Renewal Date: March 2013, 2015

W. Dean Rupp, Ph.D., Chair
Yale Biological Safety Committee

Benjamin Fontes, Biosafety Officer
Yale Office of Environmental Health & Safety

REPORTING REQUIREMENTS:

Specific reporting requirements applicable to all rDNA experiments

- **Any spill, accident, injury or exposure involving BSL2 or higher rDNA materials must immediately reported to the Biological Safety Committee and the NIH Office of Biotechnology Activities by phone (301) 496-9838; fax (301) 496-9839; E-mail: oba@od.nih.gov, or mail.**
  - **Please notify the Environmental Health & Safety Biosafety Office at 203-785-3550, or the EHS emergency line at 203-785-3555 to initiate the review and the notification process.**
- **Yale IACUC authorization is required prior to initiation of this rDNA experiment (http://iacuc.yale.edu/).**

- [ ] The Yale Biological Safety Committee did not approve your protocol at the meeting due to the following:

  - [ ] Incomplete or illegible rDNA Protocol Application
  - [ ] Additional information requested, please send the following information:
  - [ ] Insufficient training, please complete the following:
  - [ ] Lab facility and work practice inspection required, please contact the Biosafety Officer at 785-3550 to schedule an inspection.
  - [ ] Other: **State of CT Human Pathogen Authorization Required**

This box checked if your protocol has not been approved. The specific reason will also be indicted.

Enclosed is an approved registration document for your files. The general requirements for all approved rDNA protocols are included at the end of the approval letter. Your specific approval requirements are checked off in the following Section.

Additional containment requirements for your protocol will be indicated in the following

Section 2: Protocol Specific Requirements

- [ ] There are no additional protocol specific requirements for this project. Please refer to Section 3 for the general rDNA protocol requirements for this experiment.

- [ ] **Additional BSL2 Cell Culture Precautions:**
  - The facility should be self-contained, that is, all equipment needed for the experiment must be located in your laboratory;
  - All work must be done in a biosafety cabinet;
  - Full face protection is required for any procedure that may involve the manipulation of biohazards that could involve splash or splatter to the face;
  - Vacuum lines must be protected by filters;
  - Eliminate sharps, such as needles or glass Pasteur pipettes, from your experiments;
  - Use containment accessories (sealed tubes and safety canisters or rotors with sealed O-rings) for centrifugation;
  - A lab coat or back-fastening gown and gloves must be worn (face protection is required for procedures that may involve splash or splatter); and
  - Cultures should be monitored for replication competent viruses.

Required criteria for animal rDNA experiments start in this section

- [ ] Work in animals is approved at Animal Biosafety Level 2 with the requirement that researchers schedule a meeting with Biosafety and representatives from the Yale Animal Resources Center prior to initiating this work in animals. Please contact Ms. Heidi Voegeli at 785-3641 to schedule a meeting.

- [ ] An approved Institutional Animal Care and Use Committee (IACUC) Protocol is required for this experiment.

- ☐ A United States Department of Agriculture (USDA) permit is required to obtain this agent from a group outside of your laboratory (including other Yale Investigators).
- ☐ Researchers handling this agent must avoid contact with livestock (hooved animals) for at least 5 days after last handling the agent in cell culture or 5 days after last handling animals that have been inoculated with the agent.
- ☐ A successful lab inspection is required prior to the initiation of this experiment. Please contact the Biosafety Office at 785-3550 to schedule the inspection.
- ☐ New BSL2+ and BSL3 researchers also require approval by the BL3 Subcommittee before initiating independent BSL2+ or BL3 work.
- ☐ Personnel enrolled in this protocol must complete a work practice observation prior to the initiation of independent research on the project. Please contact the Biosafety Office at 785-3550 to schedule a work practice observation.
- ☐ This protocol has the following Medical Surveillance Program requirements:

Any special medical requirements will be noted here

  - ☐ As this protocol involves the use of a biohazard, immunosuppressed or immunocompromised individuals may not be assigned to this project without consultation with the Employee Health Physician. Any individual with an immunosuppressive condition may notify the Yale Employee Health Office (432-7978) for a confidential evaluation to request consideration for clearance to work on this project. If the health status of any individual assigned to this project changes after the initiation of the project, the individual must notify the Yale Employee Health Office for a medical clearance review.
  - ☐ Researchers involved in this protocol must be cleared by Yale Employee Health.
  - ☐ This project requires enrollment in the campus serum storage program.
  - ☐ There is an immunization that may be required for enrollment in this protocol and a Medical Consultation with Yale Employee Health is required.
  - ☐ Research involves a reproductive pathogen; men and women of childbearing Age should contact Yale Employee Health at 432-7978 to discuss any concerns they may have regarding work with this agent
  - ☐ This protocol involves the use of a defective Lentivirus vector. As there is a theoretical risk of a false positive result on an HIV antibody test, please immediately document all exposures with this vector with Employee Health.

Section 3: General rDNA Protocol Requirements

The following is required for all rDNA protocols approved by the Yale Biological Safety Committee.

General requirements for all rDNA Protocols are included in this final part of your approval letter

- Please ensure that all new personnel on the protocol fulfill all applicable OEHS training requirements (i.e. Biosafety, Chemical Safety, initial and annual Bloodborne Pathogens training) before initiating work on the protocol.
- It is the responsibility of the Principal Investigator to train new personnel before they begin work
- All new personnel must also demonstrate proficiency with the required work safety practices, engineering controls, and the laboratory techniques utilized in the experiment. The Principal Investigator must ensure that all researchers on this protocol have sufficient experience prior to working independently on this project.
- Should you wish to add personnel to your project, change the scope or location of your work, you must notify the Biosafety Office at 785-3550.
- A Biosafety representative will visit your laboratory periodically to monitor your facility and procedures, and to answer any questions you may have regarding safety.

13.4 NIH Definition of Synthetic Nucleic Acid Molecules

National Institutes of Health • Office of Biotechnology Activities

FREQUENTLY ASKED QUESTIONS

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

1. **Why has the NIH modified the *NIH Guidelines* to include synthetic nucleic acid molecules?**

   The impetus for amending the *NIH Guidelines* was two-fold:

   (1) recognition that appropriate biosafety containment of an agent is critical regardless of the technology used to generate that agent (i.e., recombinant or synthetic techniques), and
   (2) a recommendation by the National Science Advisory Board for Biosecurity (NSABB) that the United States Government work with the scientific community to ensure that current biosafety guidelines are appropriate, adequate, and easily understood with respect to working with synthetic nucleic acids.

2. **What has changed with respect to the scope of the *NIH Guidelines*?**

   The scope of the *NIH Guidelines* has been modified to cover explicitly certain types of basic and clinical research with nucleic acid molecules created solely by synthetic means. Certain classes of basic and clinical research with synthetic nucleic acids will be exempt.

   The new language in Section I-A of the *NIH Guidelines* states "The purpose of the *NIH Guidelines* is to specify the practices for constructing and handling:

   (i) recombinant nucleic acid molecules,
   (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and
   (iii) cells, organisms, and viruses containing such molecules."

   Throughout the *NIH Guidelines*, the term "recombinant DNA molecules" has been replaced as appropriate with "recombinant or synthetic nucleic acid molecules" which encompasses research with both recombinant and/or synthetic nucleic acids. As a result, the amended *NIH Guidelines* apply (unless otherwise exempted by other sections of the *NIH Guidelines*, e.g. III-F) to research with recombinant or synthetically derived nucleic acids, including those that are chemically or otherwise modified analogs of nucleotides (e.g., morpholinos), or both.

Synthetic Amendment FAQ/September 2012

3. **How are recombinant and synthetic nucleic acids defined under the amended *NIH Guidelines*?**

   In the amended *NIH Guidelines,* recombinant and synthetic nucleic acid molecules are defined as:

   (i) molecules that a) are constructed by joining nucleic acid molecules and b) can replicate in a living cell (i.e. recombinant nucleic acids);
   (ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e. synthetic nucleic acids); or
   (iii) molecules that result from the replication of those described in (i) or (ii) above.

4. **What institutions are subject to the amended *NIH Guidelines*?**

   Institutions that receive NIH funding for any research involving recombinant or synthetic nucleic acids, unless such research is specifically exempted by the *NIH Guidelines*, must comply. Previously, institutions were required to follow the *NIH Guidelines* only if they received NIH funding for recombinant DNA research. If an institution is receiving NIH funding for only research with synthetic nucleic acids, and that research is covered under the amended *NIH Guidelines*, any research with synthetic nucleic acids or recombinant DNA conducted at the institution, regardless of the source of funding, will need to comply with all of the requirements of the *NIH Guidelines*. For further information on institutional requirements under the *NIH Guidelines* see:
   http://oba.od.nih.gov/oba/ibc/FAQs/IBC_Frequently_Asked_Questions7.24.09.pdf/.

   Other Federal agencies, including the Departments of Energy, Veterans Affairs, and Agriculture, may have policies in place stating that recombinant or synthetic nucleic acid research conducted by or funded by these agencies must comply with the *NIH Guidelines*, and investigators receiving funding from other Government agencies need to check with those agencies regarding the applicability of the amended *NIH Guidelines*. While the *NIH Guidelines* may not govern all Government funded and privately funded research, it may be used as a tool for the entire research community to understand the potential biosafety implications of this type of research.

5. **When do the requirements under the amended *NIH Guidelines* go into effect?**

   The changes are effective March 5, 2013, which is six months from the date of publication. All ongoing and proposed experiments with synthetic nucleic acids newly subject to the amended *NIH Guidelines* need to be registered by the Principal Investigator with the Institutional Biosafety Committee (IBC) by the effective date listed above. The six month time frame was deemed sufficient to allow institutions to develop new procedures, as well as

outreach and training for investigators whose research now will be subject to the amended *NIH Guidelines*.

6. What basic research with synthetic nucleic acids is covered under the amended *NIH Guidelines*?

The amended *NIH Guidelines* apply to research with synthetic nucleic acids that presents biosafety risks equivalent to recombinant DNA research that is subject to the *NIH Guidelines*. For example, research with a genetically modified virus or a vector derived solely by synthetic techniques is subject to the amended *NIH Guidelines*.

7. What impact is this change projected to have on institutions?

Presently, most research with synthetic nucleic acids also involves the use of recombinant techniques and is already covered by the *NIH Guidelines*. Thus, the NIH anticipates that in the near-term there will not be a significant increase in the amount of research subject to the amended *NIH Guidelines* due to these amendments.

8. What basic research with synthetic nucleic acids is exempt from the amended *NIH Guidelines*?

In keeping with the exemptions for recombinant DNA molecules described in Section III-F of the previous version of the *NIH Guidelines*, certain synthetic nucleic acid molecules are exempt from the amended *NIH Guidelines* because:

(1) their introduction into a biological system is not expected to present a biosafety risk that requires review by an IBC, or

(2) the introduction of these nucleic acid molecules into biological systems would be akin to processes of nucleic acid transfer that already occur in nature, so that the appropriate biosafety practices would be the same as those used for the natural organism.

Even though these molecules are exempt from the amended *NIH Guidelines*, other federal and state standards of biosafety may still apply to research with such molecules (e.g., the Centers for Disease Control and Prevention/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*). The exemptions that were in the previous version of the *NIH Guidelines* under Section III-F also will apply to research with synthetic nucleic acids. This includes an exemption for any research with synthetic nucleic acids that are not contained in cells, organisms, or viruses.

In addition, in review of the *NIH Guidelines*, it was recognized that most biosafety risks arise from recombinant molecules because they are designed to replicate or are derived from molecules that can replicate. Synthetic nucleic acid molecules may not be designed to replicate and therefore may not pose the same biosafety risks. Also, a biosafety risk may arise due to the ability of nucleic acid molecules to integrate into the genome or produce a toxin. Therefore, an

exemption was made for research with certain low-biosafety-risk synthetic nucleic acid molecules. Specifically, synthetic nucleic acids molecules that meet the following criteria will be exempt:

Those synthetic nucleic acids that:

(1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and
(2) are not designed to integrate into DNA, and
(3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight.

An example of an exempt molecule would be research with a synthetic short-interfering RNA (siRNA) that targets an HIV viral protein required for transcription activation, even if this siRNA is injected into animals or used in cell culture.

9. Does the synthesis of a naturally occurring organism fall under the amended *NIH Guidelines*?

The *NIH Guidelines* always have exempted research with recombinant molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, although one or more segments may be a synthetic equivalent. This language exempted nucleic acid sequences that are essentially copies of those found in nature. The language has been modified in the amended *NIH Guidelines* by limiting this exemption to those nucleic acid sequences that exist contemporaneously in nature. The new exemption now applies to those molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists outside of a laboratory setting.

Research with nucleic acid sequences for organisms that do not currently exist in nature outside of the laboratory setting would not be exempt (e.g., an identical copy of the 1918 H1N1 influenza virus).

10. Is the chemical synthesis of nucleic acids subject to the *NIH Guidelines*?

No. The amended *NIH Guidelines* do not cover the chemical synthesis of nucleic acids. While the scope of the amended *NIH Guidelines* refers to "constructing" nucleic acids, the amended *NIH Guidelines* exempts research with nucleic acids that are not contained in cells, organisms, or viruses. Therefore, the chemical synthesis of nucleic acids is exempt. The amended *NIH Guidelines* only apply once synthetic nucleic acids are placed in a biological system.

11. Will recombinant DNA protocols still need to be reviewed by an Institutional Biosafety Committee?

Yes. The existing requirements to review recombinant DNA research that were previously covered under the *NIH Guidelines* have not changed.

12. Does research need to encompass both recombinant and synthetic nucleic acids to be subject to the amended *NIH Guidelines*

No. The amended *NIH Guidelines* apply to research involving either recombinant or synthetic nucleic acid molecules (or both in combination) unless the research is specifically exempted under one or more conditions listed in Section III-F.

13. What clinical research with synthetic nucleic acids is covered under the amended *NIH Guidelines*?

Section III-C-1 of the amended *NIH Guidelines* covers human gene transfer experiments, i.e., research that involves the deliberate transfer into human research participants of either:

- Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
- Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
  - Contains more than 100 nucleotides; or
  - Possesses biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
  - Have the potential to replicate in a cell; or
  - Can be translated or transcribed.

The above criteria are designed to capture synthetic nucleic acid agents that share common characteristics with current gene transfer agents that are delivered by vectors. The key characteristics of gene transfer agents are their ability to be translated or transcribed, potential for replication, even if engineered to be replication incompetent; and their ability to integrate and persist. In contrast, synthetic oligonucleotides, such as short interfering RNAs or micro-RNAs, that are directly administered or delivered in chemical vehicles, such as nanoparticles, are very small molecules and do not share these same characteristics. This definition of human gene transfer is designed to exclude these constructs from the amended *NIH Guidelines* and instead to only capture agents that would be the equivalent of recombinant gene transfer agents, (e.g., short-hairpin RNA that is delivered in a plasmid, bacterial, or other viral vector).

14. How do the changes affect what gene transfer protocols need to be registered?

OBA does not anticipate a significant increase in the number or types of gene transfer protocols that will need to be registered. At this time, recombinant techniques are predominately used to create vectors, such as a plasmid. It is likely that over the next few years or longer recombinant techniques will continue to be used to create large nucleotides that are designed to integrate, replicate, or be translated or transcribed. However, as synthetic techniques evolve, this may change and it may be more efficient to produce synthetic vectors for gene transfer.

15. How does a risk assessment for research with synthetic nucleic acid molecules differ from a risk assessment for research with recombinant nucleic acid molecules?

The risk assessment framework of the *NIH Guidelines* uses the risk group of the parent organism as a starting point for determining the necessary containment level. For example, genetic modifications of a Risk Group 3 organism (defined as agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available) would generally be carried out at Biosafety Level 3 (BL3) containment but the containment level might be raised or lowered depending on the specific construct and the experimental manipulations.

In most instances, this risk assessment framework can be effectively applied to assess the biosafety risks of experiments with synthetic nucleic acids. However, synthetic biology research has the potential to create complex, novel organisms for which identification of a parent organism may be more difficult or may not be as relevant to the risk assessment as it is with more traditional recombinant organisms. The risk assessment may also be complicated by the limitations in predicting gene function from sequence(s) or the synergistic effects from combining sequences from different sources in a novel context. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest risk group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The risk assessment should also consider that the combination of certain sequences in a new biological context may result in an organism for which the risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through the combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

16. Will the composition and expertise of IBCs need to change to address the changes to the amended *NIH Guidelines*?

No. The required composition of an IBC has not changed under the amended *NIH Guidelines*. In general, when constituting an IBC, institutions should consider their research portfolios and constitute the IBC so that it has the necessary expertise to review research that is subject to the amended *NIH Guidelines*.

17. Where can I get more information about the *NIH Guidelines*?

Further information is available on the NIH OBA website at:

http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

If you have specific questions about the *NIH Guidelines*, please contact the NIH Office of Biotechnology Activities by e-mail at oba@od.nih.gov, by telephone at 301-496-9838, by fax to 301-496-9839, or by mail to:

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750
Bethesda
Maryland 20892-7985 (20817 for non-USPS mail).

13.5 NIH OBA Exempt Experiment FAQs

National Institutes of Health ▪ Office of Biotechnology Activities

FAQs about experiments that are exempt from the *NIH Guidelines*

1. Which experiments are exempt from the *NIH Guidelines for Research Involving Recombinant DNA Molecules*?

Per Section III-F of the *NIH Guidelines*, experiments are exempt when they involve recombinant DNA that is:

- Not in organisms and viruses;
- Entirely DNA segments from a single nonchromosomal or viral DNA source;
- Entirely from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host or when transferred to another host by well established physiological means;
- Entirely from a eukaryotic host including its chloroplasts, mitochondria, or plasmids when propagated only in that host or a closely related strain of the same species,
- Entirely segments from different species that exchange DNA by known physiological processes, though one or more may be a synthetic equivalent; see Appendix A of the *NIH Guidelines*; or
- Not a significant risk to health or the environment as determined by the NIH Director, with advice from the RAC and public comment; see Appendix C of the *NIH Guidelines* for a detailed listing;

Unless these experiments also involve:

- The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture [Section III-A];
- Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram of body weight [Section III-B]; or
- The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into one or more human research subjects [Section III-C].

Details on certain other experiments that may be exempt, as well as exceptions, may be found in Appendix C of the *NIH Guidelines*.

2. The *NIH Guidelines* exempt certain experiments that do not pose a threat to health or the environment. Can an Institutional Biosafety Committee (IBC) or Principal Investigator (PI) determine if an experiment does not pose such a threat and is therefore exempt?

Section III-F-6 of the *NIH Guidelines* lists categories experiments that do not present a significant risk to health or the environment and are therefore exempt. The determination of the types of experiments that fall into this category is made by the NIH Director with the advice of the RAC, following appropriate notice and opportunity for public comment. **PIs and IBCs can not make the**

6705 Rockledge Drive, Suite 750, Bethesda MD 20892-7985 • Tel (301)496-9838 • Fax (301)496-9839
Email: oba@od.nih.gov • http://www4.od.nih.gov/oba

determination that a class of experiments other than the ones listed below poses no significant risk.

The following classes of experiments are exempt under Section III-F-6:

Please note that the information in this NIH publication was based on a previous version of the NIH Guidelines but is accurate except for the section under which the described experiments are exempt. The section is now III-F-8

- Recombinant DNA in tissue culture [Appendix C-I]
- *Escherichia coli* K-12 host-vector systems [Appendix C-II]
- *Saccharomyces* host-vector systems [Appendix C-III]
- *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems [Appendix C-IV]
- Extrachromosomal elements of gram positive organisms [Appendix C-V]
- The purchase or transfer of transgenic rodents [Appendix C-VI]

A full description of the exemptions with exceptions can be found in Appendix C of the *NIH Guidelines*.

3. How do I know if I am working with host-vector system that is exempt from the *NIH Guidelines*?

Only certain experiments that use *E.coli* K-12, *Saccharomyces*, *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems are specifically exempted from the *NIH Guidelines* (see Appendix C-II). If you are obtaining a host-vector system from a vendor, genotype information may be available and permit determination of the strain from which your host is derived.

4. DNA molecules resulting from the replication of recombinant DNA are subject to the *NIH Guidelines*. Are any other materials derived from or produced by genetically engineered organisms subject to the requirements *NIH Guidelines*?

No. For example, proteins produced by genetically engineered organisms are not subject to the *NIH Guidelines*.

5. I have heard that certain kinds of human gene transfer trials are exempted from the requirements of the *NIH Guidelines* – is this true?

No. All trials involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the *NIH Guidelines*. Appendix M-VI-A of the *NIH Guidelines* exempts certain types of vaccine trials from the requirements for **submission of the protocol to NIH OBA, RAC review, and subsequent reporting** (Appendix M-I). Specifically, this exemption applies to "human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected." Trials with these characteristics do not have to be registered with NIH OBA or undergo RAC review, but can be submitted on a voluntary basis, particularly if the investigator believes that a trial presents scientific, safety, or ethical concerns that would benefit from RAC review and public discussion. Investigators that submit trials voluntarily will be expected to comply with all aspects of the protocol review and reporting requirements. OBA encourages investigators and institutional review bodies to contact us (oba@od.nih.gov) for assistance in determining whether this exemption applies to a particular trial.

It is important to note that Appendix M-VI-A does <u>not</u> exempt these vaccine trials from other requirements specified in the NIH Guidelines, including biosafety review. Thus, vaccine trials, like other human gene transfer trials subject to the NIH Guidelines, must be reviewed and approved by an IBC before research participants can be enrolled.

6. **There is a note at the beginning of Section III of the *NIH Guidelines* that states "If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines."* What is meant by this note?**

If an experiment falls into Section III-D or III-E of the *NIH Guidelines* and <u>also</u> falls into section III-F, it is exempt. An example of such an experiment is the following:

Staphylococcus aureus (a Risk Group 2 bacterium) contains a recombinant plasmid. The plasmid is indigenous to S. aureus, was created in vitro, and contains only DNA from S. aureus (i.e., the DNA inserted into the plasmid was S. aureus DNA).

Rationale: The introduction of recombinant DNA into Risk Group 2 agents is usually covered under Section III-D-1-a. However, because the experiments are only performed in the *S. aureus strain*, this work would fall under III-F-3 (experiments that consists entirely of DNA from a prokaryotic host including its indigenous plasmids when propagated only in that host or a loosely related strain of the same species). Thus this experiment falls into both Sections III-D and III-F and is exempt, due to the above note from the requirements of the *NIH Guidelines* for IBC review and approval.

It should be noted that only experiments covered by both III-D or III-E and III-F can be exempted. If an experiment falls into Section III-A, III-B or III-C and any one of the other sections, then the rules pertaining to Sections III-A, III-B or III-C must be followed.

7. **Appendix C-1 of the *NIH Guidelines* exempts experiments involving recombinant DNA in tissue culture. I have a cell line that was created by the introduction of recombinant DNA. Are all experiments I conduct with this cell line exempt from the requirements of the *NIH Guidelines*?**

No. Although Appendix C-1 does exempt the use of recombinant DNA in tissue culture, there are exceptions to this exemption. Existing tissue culture cell lines created by the introduction of recombinant DNA are exempt from the *NIH Guidelines* unless, the cell line:

- was modified using DNA from Risk Group 3 or 4 agents, or from restricted agents. [Section III-D]
- contains a toxin with an LD50 of less than 100 ng/kg body weight. [Section III-B-1]
- contains viral DNA in a quantity exceeding 50% of any viral genome. [Appendix C-I]
- is used in conjunction with defective viruses in the presence of helper virus. [Section III-D-3]
- is used in an experiment involving the deliberate transfer of the cell line into humans. [SectionIII-C-1]
- is grown in a volume exceeding 10 liters of culture. [Section III-D-6]

13.6 NIH OSP Major Actions Under Sections III-A of the NIH Guidelines

Major Actions under Section III-A of the *NIH Guidelines* for Research Involving Recombinant or Synthetic Nucleic Acid Molecules - May 2019

1. What experiments are considered "Major Actions" under the *NIH Guidelines*?

Under the *NIH Guidelines*, the term "Major Action" means that NIH Director approval is required. Only one type of experiment requires NIH Director approval – the deliberate transfer of a drug resistance trait to a microorganism when such resistance could compromise the ability to control the disease agent in humans, veterinary medicine, or agriculture (see Section III-A-1-a of the *NIH Guidelines*).

2. What criteria should be used to determine if the transfer of a particular drug resistance trait is considered a Major Action under Section III-A-1-a of the *NIH Guidelines*?

An experiment may be considered a Major Action if: 1) it involves the use of recombinant or synthetic nucleic acids to introduce drug resistance into a microorganism, and 2) the drug in question is used to treat disease caused by the organism in humans, veterinary medicine, or agriculture. The experiment would not be considered a Major Action if there is sufficient documentation that resistance to a therapeutically useful concentration of that drug exists in the agent outside of a laboratory setting. Such evidence should be in the form of articles published in the scientific literature.

3. What is considered a therapeutically useful drug?

A drug is therapeutically useful if it is effective in the treatment of the disease caused by the microorganism. It does not have to be the 'first line' agent but should be recognized in the scientific literature as a useful drug. *In vitro* sensitivity to the drug is not sufficient; it must be useful *in vivo*.

In addition, if a drug is not therapeutically useful but will confer cross resistance to a therapeutically useful drug, then this also is considered a III-A-1-a experiment.

A drug is considered to be useful for treatment even if its use is limited to the treatment of a specific patient population (for example, children or pregnant women), or it is primarily used for treatment outside of the United States where alternative drugs are not available (e.g., chloramphenicol is not in common use in the U.S. but is used in many other countries).

4. Can an Institutional Biosafety Committee (IBC) determine if a particular experiment meets the criteria of Section III-A-1-a or must NIH make the determination?

The investigator should make an initial assessment regarding whether a particular experiment constitutes a Major Action, with confirmation by the IBC. IBCs are encouraged to contact the NIH Office of Science Policy (OSP) if they need assistance in assessing whether a specific experiment involving the deliberate transfer of a drug resistance trait falls under Section III-A-1-a and therefore requires NIH Director approval. IBCs may also consult with NIH OSP regarding experiments that raise important public

Major Actions under Section III-A of the *NIH Guidelines* for Research Involving Recombinant or Synthetic Nucleic Acid Molecules - May 2019

health issues but do not meet the criteria of Section III-A-1-a (e.g., because there is low frequency of resistance to a drug). NIH OSP will consult, as needed, with appropriate experts, as needed.

5. What are the steps in the review process for a Section III-A-1-a experiment?

The steps in the review process are 1) submission of relevant information on the proposed experiment to NIH OSP, 2) publication of the proposal in the *Federal Register* for 15 days of comment, and 3) if approved, containment conditions and any special requirements for the experiments will be stipulated by the NIH Director.

6. Does the IBC need to review the proposal before it is sent to NIH?

The IBC may initiate its review of these experiments before or after submission to and approval/denial from NIH. The IBC's final determination should occur after approval by the NIH Director, because the IBC must take into account any special conditions that the NIH Director has stipulated as a condition of approval and ensure that they are implemented. Research that meets the criteria for Section III-A-1-a cannot be initiated unless approval is granted by the NIH Director (or NIH OSP in the case of an equivalent experiment).

7. What information needs to be submitted to NIH OSP for review of research that involves the transfer of drug resistance that may ultimately be reviewed and approved by the NIH Director?

Information about the proposed experiment that NIH requires to determine whether the experiment meets the criteria of Section III-A-1-a of the *NIH Guidelines* includes technical information about the proposed transfer of drug resistance (e.g., the vector(s), gene(s) encoding the resistance, degree(s) of resistance, cross-resistance to other drugs, and other pertinent characteristics of the recombinant construct).

If the experiment is considered to be a III-A-1-a experiment, the following additional information must be submitted to NIH OSP:

- Rationale for why the work should go forward, including an assessment of how the scientific and public health benefits outweigh the potential risks for humans, animals, or agriculture.
- A discussion of whether there are alternative approaches to this research that would not involve conferring resistance to a drug that has utility in the treatment of disease caused by the organism in question. This should include a statement indicating whether the submitting investigator or others have considered any alternatives.
- A description of the proposed risk mitigation strategies that will be implemented to minimize risk to laboratory personnel as well as to the public.

Major Actions under Section III-A of the *NIH Guidelines* for Research Involving Recombinant or Synthetic Nucleic Acid Molecules - May 2019

- Minutes, if available, of any IBC discussion of the research in question (IBC review is not required prior to NIH review and NIH Director approval/denial, but a preliminary review is desirable).
- IBC contact information

In addition, NIH may also request that the following information be submitted:

- Description of the biosafety features of the room(s) in which the research will be conducted.
- Most recent inspection report(s) of the room(s) in which the research will be conducted, including any reports of biosafety equipment failures or biosafety-related problems that have occurred in these rooms in the last two years.
- Biosafety manual for the proposed work.
- Description of any additional biosafety training that laboratory personnel will receive specific to the research question.
- Description of any special occupational health requirements for the laboratory personnel involved in the research (e.g., vaccination, medical surveillance).

Submission of relevant information on a proposed Section III-A-1-a experiment should be made to NIHGuidelines@od.nih.gov

8. Who approves Section III-A-1-a experiments?

The NIH Director approves these experiments. These experiments may not proceed unless approved by the NIH Director and the IBC.

9. Once a Section III-A-I-a experiment has been approved by NIH, do equivalent experiments also need to be approved by the NIH Director?

Once a Section III-A-I-a experiment has been approved by the NIH Director, equivalent experiments may not need to follow the same approval process to determine the appropriate biosafety containment level for the work. Under Section III-B-2 of the *NIH Guidelines* (Experiments that Require NIH and IBC Approval Before Initiation), NIH may determine that a proposed experiment is equivalent to one that has previously been approved by the NIH Director as a Major Action. An experiment will be considered equivalent only if, as determined by NIH, there are no substantive differences in methodology and other pertinent information has not emerged since submission of the initial III-A-1-a experiment that would change the biosafety and/or public health considerations for the proposed experiment. If NIH makes such a determination, experiments deemed to be equivalent will not require review and approval under Section III-A-1-a. These experiments will have been approved by NIH and may proceed once approved by the appropriate IBC.

Major Actions under Section III-A of the *NIH Guidelines* for Research Involving Recombinant or Synthetic Nucleic Acid Molecules - May 2019

10. My research involves the transfer of a drug resistance trait into an organism on the Department of Health and Human Services (HHS) and/or the United States Department of Agriculture (USDA) Select Agent list (i.e. is a "restricted experiment") and thus requires approval from those agencies. Do I also need to submit a request to NIH for approval?

Experiments utilizing recombinant or synthetic nucleic acid molecules that involve the deliberate transfer of a drug resistance trait into a Select Agent (that meet the definition of a restricted experiment) may be subject to the regulatory authority of, and review by, HHS and/or USDA under their respective rules (found in 42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121). Review and approval by the appropriate Federal agency (HHS or USDA) supersedes the requirement for approval under the NIH Guidelines. However, other provisions of the NIH Guidelines for example, IBC review and approval are still applicable.

11. I am conducting research that involves the transfer of resistance to certain drugs in a strain of a Select Agent that has been excluded or exempted from the Select Agent regulations. From which agency do I need to get approval?

Potential III-A-1-a experiments in strains of Select Agents that have been excluded or exempted from the Select Agent regulations should be submitted to NIH OSP.

12. Useful Links and Resources Regarding the Control of Disease Agents

Government Resources

PubMed:
http://www.ncbi.nlm.nih.gov/sites/entrez/

Centers for Disease Control and Prevention:
http://www.cdc.gov/

World Health Organization:
http://www.who.int/en/

Medline:
http://www.nlm.nih.gov/medlineplus/

Private Resources (fees may apply)

Up to Date Online
http://www.uptodateonline.com/utd/index.do

Control of Communicable Diseases Manual
Editor: David L. Heymann Publisher: American Public Health Association
ISBN-13: 978-0875530345

Johns Hopkins ABX Guide
http://www.hopkins-abxguide.org/

Red Book: Report of the Committee on Infectious Diseases
Publisher: American Academy of Pediatrics ISBN-13: 978-1581101942

13.7 NIH OBA Transgenic Animals and rDNA Use in Animals

FAQs for Research on Genetically Modified (Transgenic) Animals – May 2019

1. Under which section of the *NIH Guidelines* does the generation of transgenic rodents fall?

The creation of transgenic rodents falls under one of two sections of the *NIH Guidelines* depending on the containment level required to house the rodents. Experiments involving the creation of transgenic rodents that can be housed under Biosafety Level 1 conditions are covered under Section III-E-3. Experiments involving the generation of transgenic rodents requiring BL2, BL3 and BL4 containment are covered under Section III-D-4.

2. Under which section of the *NIH Guidelines* does the generation of transgenic animals other than rodents fall?

The creation of all transgenic animals (other than rodents that can be housed under BL1 containment conditions) is covered under Section III-D-4 of the *NIH Guidelines*.

3. Would the breeding of two different lines of knock-out mice require IBC approval under the *NIH Guidelines*?

If the technique used to create the knock-out mice involves the stable introduction of recombinant or synthetic nucleic acid molecules into the animal's genome, the animals will be considered transgenic. As the breeding of two different lines of knock-out animals will potentially generate a novel line of transgenic animal, the work may be covered under the *NIH Guidelines* and require IBC review and approval. Sections in the *NIH Guidelines* that cover work with rodents include III-E-3 for work that requires Biosafety Level (BL) 1 containment and III-D-4 for work that requires BL2, BL3 and BL4 containment. Certain breeding experiments are exempt under Appendix C-VIII of the *NIH Guidelines*. This exemption covers the breeding of two different lines of transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new line of transgenic rodent that can be housed at BL1 if:

1. both parental rodents can be housed under BL1 containment; and
2. neither parental transgenic rodent contains the following genetic modifications:
   - incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or
   - incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
3. the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

FAQs for Research on Genetically Modified (Transgenic) Animals – May 2019

4. Is IBC registration and approval needed for the maintenance of a transgenic animal colony?

The maintenance of a transgenic rodent colony (i.e. breeding within a particular transgenic line) that can be housed at BL1 is an activity that is exempt from the *NIH Guidelines* and, as such, does not require IBC registration and approval. The maintenance of a transgenic rodent colony at BL2 or higher falls under Section III-D-4-b and requires IBC approval. The breeding of all other transgenic animals is subject to the *NIH Guidelines* under Section III- D-4-a or III-D-4-b depending on the containment level required.

5. Is the purchase and transfer of transgenic rodents exempt from the NIH Guidelines?

Under Appendix C-VII of the *NIH Guidelines*, the purchase or transfer of transgenic rodents that may be housed under BL1 containment are activities that are exempt from the *NIH Guidelines*. The purchase or transfer of transgenic rodents that require BL2 or higher containment is not exempt from the *NIH Guidelines*. These animals are covered under Section III-D-4, and purchase and transfer of such animals requires IBC registration and approval.

It should be noted that the subsequent use of transgenic rodents may not be exempt from the *NIH Guidelines*. Experiments using transgenic rodents at BL1 are exempt from the *NIH Guidelines* if the research does not involve the use of recombinant or synthetic nucleic acid molecules. If the protocol does involve the use of recombinant or synthetic nucleic acid molecules, then the work falls under III-D-4 of the *NIH Guidelines* and as such requires IBC review and approval prior to initiation.

6. Is the purchase and transfer of transgenic animals other than rodents exempt from the *NIH Guidelines*?

No, only the purchase or transfer of transgenic rodents that may be maintained at BL1 containment is exempt from the *NIH Guidelines*. The purchase or transfer of any other animal for research purposes at any biosafety level (including BL1) is not exempt, nor is the purchase and transfer of transgenic rodents that require BL2 or higher containment.

7. Are gene ablation studies covered by the *NIH Guidelines*?

The answer to this question depends on the technique employed in the study. If recombinant techniques are used to knock out the gene, then the work would be covered under the *NIH Guidelines*.

8. Who has the responsibility to review the generation of transgenic animals if an institution is generating animals for investigators who are not affiliated with that institution?

The generation (creation) of transgenic animals is an activity covered under the *NIH Guidelines*. The IBC at the institution where that activity is occurring has the responsibility to review and approve that activity (if the institution is subject to the requirements of the *NIH Guidelines*). The subsequent use of the animals by an investigator not at that institution would need to be reviewed and approved by the IBC at that investigator's institution if that institution conducts or supports research with recombinant or synthetic nucleic acid molecules, receives NIH support for such research, and the activity is covered under the *NIH Guidelines*.

FAQs for Research on Genetically Modified (Transgenic) Animals – May 2019

9. When a core facility generates transgenic mice as a "fee for service" for a Principal Investigator (PI), is it the responsibility of the PI or the core facility to register the generation of the mice with the IBC?

Section IV-B-7-a-(1) of the *NIH Guidelines* articulates one of the responsibilities of the PI as 'initiating no recombinant or synthetic nucleic acid molecules research which requires IBC approval prior to initiation until that research has been approved by the IBC and has met all other requirements of the *NIH Guidelines*.' It would be acceptable for either the PI of the core facility or the PI purchasing the transgenic animals to fulfill the responsibility to register the generation of the animals. In many cases, the animals being generated will be subsequently used in experiments that are subject to the *NIH Guidelines*, and the registration of the research with the IBC may encompass both the generation and subsequent experimentation with the animals.

10. When existing transgenic animals at an institution are purchased or transferred to an investigator outside the institution, who should review and approve the use of these animals?

An institution's IBC does not need to review and approve the use of transgenic animals at another institution. If the receiving institution is subject to the *NIH Guidelines* (i.e. receives NIH support for research with recombinant or synthetic nucleic acid molecules), then the purchase and transfer of animals (other than rodents that can be housed under BL1 containment), along with any experiments subject to the *NIH Guidelines*, would require review and approval by the IBC at that institution.

11. What are the *NIH Guidelines* requirements for research with large transgenic animals (sheep, pigs, etc.), or research with recombinant or synthetic nucleic acid- modified microorganisms in such animals?

When conducting research with recombinant or synthetic nucleic acid molecules in large animals, the work is covered under Appendix M of the *NIH Guidelines*. Appendix M specifies containment and confinement practices when animals are of a size or have growth requirements that preclude the use of laboratory containment of animals. The *NIH Guidelines* include provisions for tracking and inventorying these animals (Appendix M**–**1-B-2 states that a permanent record must be maintained of the experimental use and disposal of each animal). Animal carcasses must be disposed of as to avoid their use as food for human beings or animals unless food use is specifically authorized by an appropriate federal agency (Appendix M**–**1-B-1). An acceptable method, for example, would be incineration.

FAQs for Research on Genetically Modified (Transgenic) Animals – May 2019

12. Are recombinant or synthetic nucleic acid modifications to the somatic cells of nontransgenic animals subject to the *NIH Guidelines*?

Yes, these experiments are subject to the *NIH Guidelines*.

- Sections III-D-1-a through III-D-1-d cover experiments using Risk Group 2, 3, 4, or restricted agents in whole animals. See the *NIH Guidelines* for the appropriate containment for such experiments
- Section III-D-4-a covers experiments involving viable recombinant or synthetic nucleic acid-modified microorganisms tested on whole animals. Recombinant or synthetic nucleic acid from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any animal and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study.
- Section III-D-4-b covers recombinant or synthetic nucleic acid, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals that are not covered by Sections III-D-1 or III-D-4-a. The appropriate containment for these experiments is determined by the IBC.
- Experiments not included in Sections III-A, III-B, III-C, III-D, III-F, fall into Section III-E. Experiments covered by Section III-E may be conducted at BL1 containment.

For further information about the requirements of the *NIH Guidelines*, please email: NIHGuidelines@od.nih.gov.

13.8 Animal Experiments Covered Under the NIH Guidelines

Animal experiments covered under the
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

ACTIVITY	MINIMUM BSL	SECTION
CREATION OF TRANSGENIC ANIMALS		
Creation of transgenic rodents	BL1	III-E-3
Creation of transgenic rodents	BL2 or higher	III-D-4-b
Creation of transgenic animals other than rodents	BL1/BL1-N	III-D-4-a
Creation of transgenic animals other than rodents	BL2/BL2-N or higher	III-D-4-b
Creation of recombinant or synthetic nucleic acid molecule modified arthropods	BL1	III-D-4-a
Creation of recombinant or synthetic nucleic acid molecule modified arthropods	BL2 or higher	III-D-4-b
Creation of knock-out rodents	BL1	III-E-3
Creation of knock-out rodents	BL2 or higher	III-D-4-b
BREEDING OF TRANSGENIC ANIMALS		
Breeding rodents from one strain (propagation/colony maintenance)	BL1	Exempt
Breeding rodents from one strain (propagation/colony maintenance)	BL2 or higher	III-D-4-b
Breeding rodents from two strains (generating a new strain) providing neither parental rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and (3) the rodent that results from the breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.	BL1	Exempt (Appendix C-VIII)
Breeding rodents from two strains (generating new strain) if the parental rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); or (3) the rodent that results from the breeding contains more than one-half of an exogenous viral genome from a single family of viruses.	BL1	III-E-3
Breeding rodents from two strains (generating new strain)	BL2 or higher	III-D-4
Breeding of transgenic animals other than rodents	BL1	III-D-4
Breeding of transgenic animals other than rodents	BL2 or higher	III-D-4
Breeding of recombinant or synthetic nucleic acid molecule modified arthropods	BL1	III-D-4
Breeding of recombinant or synthetic nucleic acid molecule modified arthropods	BL2 or higher	III-D-4
Breeding of knockout rodents from one strain (propagation/ colony maintenance)	BL1	Exempt
Breeding of knockout rodents from two strains (propagation/colony maintenance)	BL2 or higher	III-D-4

Breeding of knockout rodents from two strains (generating new strain) providing neither parental rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and (3) the rodent that results from the breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.	BL1	Exempt (Appendix C-VIII)
Breeding of knockout rodents from two strains (generating new strain) if the parental rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); or (3) the rodent that results from the breeding contains more than one-half of an exogenous viral genome from a single family of viruses	BL1	III-E-3
Breeding of knockouts from two strains (generating new strain)	BL2 or higher	III-D-4-b
EXPERIMENTS WITH TRANSGENIC ANIMALS		
Experiments with transgenic rodents	BL1	III-D-4-a* (see note)
Experiments with transgenic rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with transgenic animals other than rodents	BL1	III-D-4-a
Experiments with transgenic animals other than rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with recombinant or synthetic nucleic acid molecule modified arthropods associated with plants	BL1	III-E-2-b-(5)
Experiments with recombinant or synthetic nucleic acid molecule modified arthropods associated with plants	BL2 or higher	III-E-2
Experiments with recombinant or synthetic nucleic acid molecule modified arthropods not associated with plants	BL1	III-D-4-a
Experiments with recombinant or synthetic nucleic acid molecule modified arthropods not associated with plants	BL2 or higher	III-D-4-b

*The purchase or transfer of transgenic rodents requiring BL1 containment is exempt under Appendix C-VI. Subsequent use of these animals is also exempt providing the experimental protocol does not involve the use of recombinant or synthetic nucleic acid molecules. If the protocol does involve the use of recombinant or synthetic nucleic acid molecules then the research is covered under III-D-4-a. All experiments involving the use of other transgenic animals at any Biosafety Level and the use of transgenic rodents requiring BL2 or higher containment are subject to the *NIH Guidelines*. See above for applicable sections.

EXPERIMENTS WITH RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES IN AN ANIMAL (TRANSGENIC OR OTHERWISE)		
Experiments with recombinant or synthetic nucleic acid molecule modified microbes in any animal (transgenic or otherwise)	BL1/BL1-N	Not permitted at BL1*
Experiments with RG2 recombinant or synthetic nucleic acid molecule modified microbes in any animal (transgenic or otherwise)	BL2/ BL2-N	III-D-1-a
Experiments with RG3 recombinant or synthetic nucleic acid molecule modified microbes in any animal (transgenic or otherwise)	BL3/ BL3-N	III-D-1-b
Experiments with RG4 recombinant or synthetic nucleic acid molecule modified microbes in any animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-c
Experiments with recombinant or synthetic nucleic acid molecule modified restricted agent in an animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d

Experiments with recombinant or synthetic nucleic acid molecule modified animal pathogens in an animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Introduction of less than 2/3 of eukaryotic viral genome into a non-human vertebrate or invertebrate	BL1/BL1-N	III-D-4-a
Propagation of animals containing viral vector sequences not leading to transmissible infection	BL1/BL1-N	III-D-4-a
Experiments with recombinant or synthetic nucleic acid molecules involving whole animals not covered by Sections III-D-1or III-D-4-a	Set by IBC	III-D-4-b
* Other than viruses which are only transmitted vertically, the experiments may not be conducted at BL1. A minimum of BL2 or BL2-N is required.		
CLONING ANIMALS		
Cloning animals	BL1 or higher	Not covered
PURCHASE OR TRANSFER OF TRANSGENIC ANIMALS		
Purchase or transfer of transgenic rodents	BL1	Exempt (Appendix C-VII)
Purchase or transfer of transgenic rodents	BL2 or higher	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL1	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL2 or higher	III-D-4
Purchase or transfer of recombinant or synthetic nucleic acid molecule modified arthropods	BL1	III-D-4
Purchase or transfer of recombinant or synthetic nucleic acid molecule modified arthropods	BL2 or higher	III-D-4
PLANT EXPERIMENTS WITH ANIMALS OR ARTHROPODS		
Experiments with microorganisms or insects containing recombinant or synthetic nucleic acid molecules with the potential for detrimental impact to ecosystems.	BL3-P or BL2-P plus biological containment	III-D-5-a or III-D-5-b
Experiments with exotic infectious agents in the presence of arthropod vectors	BL4-P	III-D-5-c
Experiments with microbial pathogens of insects or small animals associated with plants with the potential for detrimental impact to ecosystems.	BL3-P or BL2-P plus biological containment	III-D-5-e
Small animals associated with recombinant or synthetic nucleic acid molecule modified plants.	BL1	III-E-2
Experiments with recombinant or synthetic nucleic acid molecule modified arthropods or small animals associated with plants.	BL1	III-E-2-b-(5)
OTHER		
Transfer of a drug resistance to microorganisms compromising the use in veterinary medicine	Set by NIH (case by case)	III-A-1-a

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*) are available at:

https://osp.od.nih.gov/biotechnology/nih-guidelines/

For further information about the requirements of the *NIH Guidelines*, please e-mail: NIHGuidelines@od.nih.gov

13.9 NIH OSP FAQs on Incident Reporting

Incident Reporting – May 2019

1. What kinds of incidents involving research subject to the NIH Guidelines must be reported to the NIH OSP?

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) states that "…any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses" must be reported to NIH within 30 days. Certain types of accidents must be reported on a more expedited basis. Spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported to NIH. Spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately reported to NIH. Relevant incidents would include spills and accidents which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules in the laboratory, rather than serious adverse events that may occur in the conduct of human gene transfer research.

2. How serious must a problem be to warrant reporting to NIH OSP?

Any spill or accident involving recombinant or synthetic nucleic acid molecule research of the nature described above or that otherwise leads to personal injury or illness or to a breach of containment must be reported to NIH OSP. These kinds of events might include skin punctures with needles containing recombinant or synthetic nucleic acid molecules, the escape or improper disposition of a transgenic animal, or spills of high-risk recombinant or synthetic materials occurring outside of a biosafety cabinet. Failure to adhere to the containment and biosafety practices articulated in the NIH Guidelines must also be reported to NIH OSP.

Minor spills of low-risk agents not involving a breach of containment that were properly cleaned and decontaminated generally do not need to be reported. NIH OSP should be consulted if the Institutional Biosafety Committee (IBC), investigator, or other institutional staff are uncertain whether the nature or severity of the incident warrants reporting; NIH OSP can assist in making this determination.

3. Who is responsible for reporting incidents involving research subject to the NIH Guidelines to NIH OSP?

Under the NIH Guidelines incident reporting is articulated as a responsibility of the Institution, IBC, Biological Safety Officer, and Principal Investigator. Institutions have the discretion to determine which party should make these reports, and one report for each incident or set of information is generally sufficient.

NIH Office of Science Policy

Incident Reporting – May 2019

4. What information should incident reports include?

Incident reports should include sufficient information to allow for an understanding of the nature and consequences of the incident, as well as its cause. A detailed report should also include the measures that the institution took in response to mitigate the problem and to preclude its reoccurrence. An incident reporting template is available to facilitate reporting of incidents under the NIH Guidelines. The template may be found on the NIH OSP website. Use of the template is not required and other report formats may be acceptable.

5. What does NIH OSP do with this information?

NIH OSP staff review incident reports to assess whether the institutional response was sufficient. Depending on the adequacy of the institutional response, NIH OSP may ask the institution to take additional measures as appropriate to promote safety and compliance with the NIH Guidelines.

6. Where should incident reports be sent?

Reports of incidents can be emailed to NIHGuidelines@od.nih.gov.

7. Where can I get more information about the NIH Guidelines?

Questions about the *NIH Guidelines* may be directed to NIH OSP staff at NIHGuidelines@od.nih.gov. Staff may also be reached at (301) 496-9838.

The incident reporting template is available at: Incident Reporting Template – 2019

NIH Office of Science Policy

13.10 NIH OBA Investigator Responsibilities Brochure

What are the *NIH Guidelines for Research Involving Recombinant DNA Molecules*?

The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) detail procedures and practices for the containment and safe conduct of various forms of recombinant DNA research, including research involving genetically modified plants and animals, and human gene transfer.

Who must comply with the *NIH Guidelines*?

All institutions that receive NIH funding for recombinant DNA research must comply with the *NIH Guidelines*. Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if their individual projects are not funded by NIH.

What is an Institutional Biosafety Committee?

Institutional Biosafety Committees (IBCs) provide local review and oversight of nearly all forms of research utilizing recombinant DNA. They ensure that recombinant DNA research conducted at or sponsored by the institution is in compliance with the *NIH Guidelines*. A requirement of the *NIH Guidelines* is that an IBC must review and approve all research subject to the *NIH Guidelines*.

What is the NIH Office of Biotechnology Activities?

The NIH Office of Biotechnology Activities (OBA) promotes science, safety, and ethics in biotechnology through the advancement of knowledge, enhancement of public understanding, and development of sound public policies. A core responsibility of OBA is to foster awareness of, and adherence to, the standards and practices set forth in the *NIH Guidelines*.

Safety and science go hand in hand

Principal Investigator Responsibilities

Principal Investigators (PIs) are responsible for full compliance with the *NIH Guidelines* during the conduct of recombinant DNA research. As part of this general responsibility, the PI should:

- Be adequately trained in good microbiological techniques.
- Provide laboratory research staff with protocols describing potential biohazards and necessary precautions.
- Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents.
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- Supervise laboratory staff to ensure that the required safety practices and techniques are employed.
- Correct work errors and conditions that may result in the release of recombinant DNA materials.
- Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment (e.g., purity and genotypic and phenotypic characteristics).
- Comply with permit and shipping requirements for recombinant DNA molecules.
- Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination.

Before initiating research subject to the *NIH Guidelines*, the PI must:

- Determine whether the research is subject to Section III-A, III-B, III-C, III-D, or III-E of the *NIH Guidelines*.
- Propose physical and biological containment levels in accordance with the *NIH Guidelines* when registering research with the IBC.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.
- Submit a research protocol to the IBC for review and approval.
- Seek OBA's determination of containment for experiments that require case-by-case review.
- Petition OBA, with notice to the IBC, for proposed exemptions from the *NIH Guidelines*.
- Obtain IBC approval before initiating research subject to the *NIH Guidelines*.
- Seek NIH approval, in addition to IBC approval, to conduct experiments specified in Sections III-A and III-B of the *NIH Guidelines*.

While conducting research subject to the *NIH Guidelines*, the PI must:

- Determine the need for IBC review before modifying recombinant DNA research already approved by the IBC.
- Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval.
- Remain in communication with the IBC throughout the duration of the project.
- Report any significant problems pertaining to the operation and implementation of containment practices and procedures, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the IBC, OBA, and, as applicable, the Biological Safety Officer, Greenhouse or Animal Facility Director, and other appropriate authorities.

14 Shipping

Shipments of research materials may be regulated by many regulatory agencies including:

- United States Department of Transportation (DOT)
- International Civil Aviation Organization (IACAO)
- International Air Transport Association (IATA)
- United States Department of Commerce (DoC) – Exports
- Centers for Disease Control and Prevention (CDC) – Imports
- United States Department of Agriculture (USDA) – Imports and transfers with in the US

To facilitate compliance with the regulations, Yale has an express shipping tool, eShipGlobal that incorporates various compliance checks. All research material shipments must be processed through eShipGlobal. Anyone involved in the shipment of research materials must have detailed knowledge of the material being shipped. Because of this, administrative staff cannot process research material shipments.

14.1 Training

14.1.1 Biological Materials and Dry Ice

Trained research staff can ship biological materials and dry ice. There are various training requirements based on the material being shipped. A training matrix is available at the following web site that outlines the training requirements based on the material being shipped:
http://ehs.yale.edu/research-materials-shipping-training-requirements.

14.1.2 Chemical and Radioactive Materials

Since the training requirements for shipping regulated chemicals (except dry ice) and radioactive materials are extensive, EHS has a Shipping team that will ship these materials once the shipment is processed through eShipGlobal. If an EHS Shipping Team member evaluates the shipment and determines the material is not regulated, the shipment will be approved and the researcher may ship the material.

14.2 Packaging

When shipping cultures or stocks of material infectious to humans or animals United Nations (UN) approved packaging is required. For information on this packaging please contact EHS at (203) 785-3550.

Packaging for human or animal materials is available at the Medical School and KBT Stockrooms. Additional packaging options are also available through SciQuest the "Research Materials Shipping Supplies" folder in the "Favorites" section of SciQuest.

14.3 Documentation

eShipGlobal will provide completed documentation and labels for research materials shipments including the air waybill, address labels, dry ice label if applicable, Shipper's Declaration for Dangerous Goods if applicable, packaging instructions, and the commercial invoice for international shipments.

14.4 Transport of Research Materials between Yale Campuses or Off Yale Campus

Research materials may not be transported on any of the Yale shuttles, public transportation, or in personal vehicles. Please contact EHS if you need to transport materials between Yale campuses.

14.5 Exports and Imports

14.5.1 Exports

The eShipGlobal system will assist in determining if an export license is required based on the material being shipped, the recipient, recipient institution, or destination country. If an export license is required,

EH&S staff will apply for the license on behalf of the PI. For additional information and resources please see http://ehs.yale.edu/exporting-research-materials.

14.5.2 Imports

Both the CDC and the USDA have import permit requirements. A table with general import permit information is on the next page. Additional information may be found at http://ehs.yale.edu/importing-research-materials. A table of import permits is on the next page. For assistance, please contact the Biosafety Office at 785-3550 or the appropriate agency.

14.5.3 Table of Required Permits

Agency	Material to be Imported
CDC Call (404) 639-3883 or visit the CDC web site at http://www.cdc.gov/od/eaipp/ for further information	**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.
	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.
	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.
	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.
	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.
	Bats - All live bats. Bats may also require a permit from the U.S. Department of Interior, Fish and Wildlife Services.
USDA Call (410) 436-8226 or visit the USDA web site at http://www.aphis.usda.gov/permits/index.shtml for further information	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. 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. Foreign plant pests injurious to plants grown in the United States. Designated noxious weeds, which are of foreign origin and new to or not widely prevalent in the United States. Insects, Mites, and Nematodes Introduced for Biological Control of Weeds in the United States. Biological control organisms imported, shipped, and released in the United States. Insects and Mites Commonly Included in Shipments as Host Material for Biological Control Agents. Domestic Plant Pests Regulated by Federal or State Quarantines. Low-Risk Organisms, including Arthropods and Pathogens. Non-regulated domestic plant pests shipped into an area in the United States where the pests do not occur.
USFWS (U.S. Fish & Wildlife Service) Call (800) 358-2104 or visit the USFWS web site at http://www.fws.gov/permits/ for further information	Certain live animals and all live bats

Appendix A 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 the 5th edition of the BMBL revised December 2009 for additional information please contact the Biosafety Office at 785-3550.

Section III—Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term "containment" is used in describing safe methods, facilities and equipment for managing infectious materials 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. The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practices 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 the 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 exposures to these hazards. Personnel should be advised of special hazards and should be required to read and 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 be responsible for the conduct of work with any infectious agents or materials. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment.

When standard laboratory practices are not sufficient to control the hazards 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 hazards associated with the agent or procedure.

Appropriate facility design and engineering features, safety equipment, and management practices must supplement laboratory personnel, safety practices, and techniques.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Safety equipment includes BSCs, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious droplets or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II BSCs are primary barriers that offer significant levels of protection to laboratory personnel and to 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 aerosol hazards, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents.

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 BSCs and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, 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 and Construction (Secondary Barriers)

The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory directors are 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 BSL-1 and BSL-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.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the ASHRAE Laboratory Design Guide published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).[1]

Biosafety Levels

Four BSLs are described in Section 4, 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 the laboratory function or activity. The BSLs described in this manual should be differentiated from Risk Groups, as described in the NIH Guidelines and the World Health Organization Laboratory Biosafety Manual. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

The recommended biosafety level(s) for the organisms in Section VIII (Agent Summary Statements) represent those conditions under which the agent ordinarily can be safely handled. Of course, not all of the organisms capable of causing disease are included in Section VIII and an institution must be prepared to perform risk assessments for these agents using the best available information. Detailed information regarding the conduct of biological risk assessments can be found in Section II. The laboratory director is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution's Biological Safety Officer (BSO) and IBC can be of great assistance in performing and reviewing the required risk assessment. At one point, under the NIH Guidelines, BSOs were required only

when large-scale research or production of organisms containing recombinant DNA molecules was performed or when work with recombinant DNA molecules was conducted at BSL-3 or above. IBCs were required only when an institution was performing non-exempt recombinant DNA experiments. Today, however, it is strongly suggested that an institution conducting research or otherwise working with pathogenic agents have a BSO and properly constituted and functioning IBC. The responsibilities of each now extend beyond those described in the NIH Guidelines and depend on the size and complexity of the program.

Generally, work with known agents should be conducted at the biosafety level recommended in Section VIII. When information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified. Often an increased volume or a high concentration of agent may require additional containment practices.

Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis*, *Nigeria gruberi*, infectious canine hepatitis virus, and exempt organisms under the NIH Guidelines are representative of 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 that have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

BSL-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 facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are 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, HIV, the *Salmonella*, and *Toxoplasma* are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard[2] 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 caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 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 in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

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

Biosafety Level 3 practices, safety equipment, and facility design and construction 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 the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate

to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-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 ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4.

The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, procedures being conducted and the nature or function of the laboratory may further influence the director in applying these recommendations.

Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. 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.

One additional biosafety level, designated BSL-3-Agriculture (or BSL 3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL 3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL 3-Ag facilities and USDA High Consequence Pathogens is provided in Appendix D.

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 that 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 serological identification of isolates can be done safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL-2 are consistent with the OSHA standard, "Occupational Exposure to Bloodborne Pathogens.[2,3] This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal or Standard* Precautions).[4,5] Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards).[6]

BSL-2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as BSCs (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limited or restricted 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 B Classification of Human Etiologic Agents on the Basis of Hazard

This section has been reprinted from the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2019.

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; Biosafety Manager, National Animal Disease Center, U.S. Department of Agriculture - ARS, Ames, Iowa 50010, Phone: (515) 337-7772.

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); adeno- associated virus (AAV – all serotypes); and recombinant or synthetic AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen); and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.

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 which 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*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
--*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
--*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
--**Francisella tularensis* specifically **F. tularensis* subspecies *novicida* [aka *F. novicida*], strain Utah 112; **F. tularensis* subspecies *holarctica* LVS; **F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)
*For research involving high concentrations, BL3 practices should be considered (see Appendix G-II-C-2. Special Practices (BL3)).
--*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*
--*Pseudomonas aeruginosa*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. choleraesuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*

--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahaemolyticus, V. vulnificus*
--*Yersinia enterocolitica*
--*Yersinia pestis* specifically *pgm*$^{(-)}$ strains (lacking the 102 kb pigmentation locus) and *lcr*$^{(-)}$ strains (lacking the LCR plasmid)

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*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*
--*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. granulosis, 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. cynomolgi, 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
--Chikungunya vaccine strain 181/25
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
--Western equine encephalomyelitis virus

Arenaviruses
--Junin virus candid #1 vaccine strain
--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*)

Caliciviruses

Coronaviruses

Flaviviruses - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Japanese encephalitis virus strain SA 14-14-2
--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 (except those listed in Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*)
--Tick-borne orthomyxoviruses

Papilloma viruses
--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 non exotic strains: VSV-Indiana 1 serotype strains (e.g.Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (*e.g.* Ogden, Hazelhurst)

Rubivirus (Togaviruses)
--Rubella virus

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 (*except the Phase II, Nine Mile strain listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
--*Francisella tularensis (*except those strains listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
--*Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
--*Orientia tsutsugamushi* (was *R. tsutsugamushi*)
--*Pasteurella multocida* type B -"buffalo" and other virulent strains
--*Rickettsia akari*, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R, siberica*, *R. typhi* (*R. mooseri*)
--*Yersinia pestis (*except those strains listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*

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
--Chikungunya virus (except the vaccine strain 181/25 listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
--Semliki Forest virus
--St. Louis encephalitis virus
--Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see Appendix B-II-D (RG2) – Viruses)
--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

Coronaviruses
--SARS-associated coronavirus (SARS-CoV)
--Middle East respiratory syndrome coronavirus (MERS-CoV)

Flaviviruses - Group B Arboviruses

--Japanese encephalitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)
--West Nile virus (WNV)
--Yellow fever virus
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Orthomyxoviruses
-- Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses
--Monkeypox virus

Prions
--Transmissible spongiform encephalopathies (TSE) 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 (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)

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 (except the candid #1 vaccine strain listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
--Machupo virus
--Sabia

Bunyaviruses (Nairovirus)
--Crimean-Congo hemorrhagic fever virus

Filoviruses
--Ebola virus
--Marburg virus

Flaviruses - 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 (Hendra virus)

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

Papilloma viruses
--Bovine papilloma virus
--Shope papilloma virus

Polyoma viruses
--Polyoma 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 BSL2+ Work Practices

Biosafety Level 2 Plus (BSL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BSL2 procedures. Generally, BL3 practices are mandated in a space designed for BSL2 work. It is preferred that the BSL2 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 BSL2+ 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 BSL2 or BSL1 usage.

BL3 practices require that all work be conducted under physical containment. Therefore, all manipulations with BSL2+ 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 BSL2+.

Personal Protective Equipment (PPE)

- Dedicate PPE for the experiment. PPE worn for BSL2+ 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 BSL2+ 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 extenders, 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 needle box 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 BSL2+, 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 < ¾ 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 an 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 BSL2+ 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 BSL2 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 it into the secondary container.

Decontaminate the secondary container before leaving the laboratory.

Research materials may not be transported on any of the Yale shuttles, public transportation, or in personal vehicles. Please contact EHS if you need to transport materials between Yale campuses.

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 D Dual Use Research of Concern

Yale *Environmental Health & Safety*

135 College Street, Suite 100
New Haven, CT 06510-2483
T 203 785-3550 F 203 785-7588
www.yale.edu/ehs

Approved by Office of the Provost – September 14, 2015

**POLICY FOR LIFE SCIENCES DUAL USE RESEARCH OF CONCERN
YALE UNIVERSITY**

I. PURPOSE

The purpose of this Policy is to strengthen the institutional review and oversight by Yale University ("Yale" or the "University") of certain research to identify potential Dual Use Research of Concern (DURC) and to develop and implement risk mitigation where appropriate. In so doing, this Policy seeks to preserve the benefits of life sciences DURC research while minimizing the risk that the output of such research would be used for harmful purposes.

This Policy sets the rules for the individuals and committees at Yale who are responsible for the implementation of the University's requirements with respect to DURC.

All research conducted at the University involving DURC Agents (as defined below) is subject to this Policy, regardless of the source of funding.

II. DEFINITIONS

BSC: The Yale University Biosafety Committee, which also serves as Yale's Institutional Biosafety Committee ("IBC")

Dual Use Research: research conducted for legitimate purposes that can be utilized for both benevolent and harmful purposes

DURC: Dual Use Research of Concern, meaning life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security

DURC Agents: the following 15 agents and toxins referred to in the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (the "2014 Policy"):

1. Avian influenza virus (highly pathogenic)
2. *Bacillus anthracis*

3. Botulinum neurotoxin (For purposes of this Policy, there are no exempt quantities of botulinum neurotoxin. Research involving any quantity of botulinum neurotoxin should be evaluated for DURC potential.)
4. *Burkholderia mallei*
5. *Burkholderia pesudomallei*
6. Ebola virus
7. Foot-and-mouth disease virus
8. *Francisella tularensis*
9. Marburg virus
10. Reconstructed 1918 Influenza virus
11. Rinderpest virus
12. Toxin-producing strains of *Clostridium botulinum*
13. Variola major virus
14. Variola minor virus
15. *Yersinia pestis*

Experimental Effects of Concern: the following 7 categories of experiments referred to in the 2014 Policy:

1. Enhances the harmful consequences of the agent or toxin
2. Disrupts immunity or effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification
3. Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies
4. Increases the stability, transmissibility or the ability to disseminate the agent or toxin.
5. Alters the host range or tropism of the agent or toxin
6. Enhances the susceptibility of a host population to the agent or toxin
7. Generates or reconstitutes an eradicated or extinct agent or toxin listed in the definition of DURC Agents above.

ICDUR: Institutional Contact for Dual Use Research, who is the individual designated by the University to be the institutional point of contact for questions relating to compliance with this Policy and the liaison with the relevant US Government funding agencies. The University has designated the Director of Environmental Health and Safety as the ICDUR.

IRE: Institutional Review Entity.
USG: US Government.

US Funding Agency: the USG agency that is funding the subject research or, if the research is not USG-funded, the USG agency designated by the NIH, based on the nature of the research. If a federal department or agency simply passes through funding from another federal department or agency to support life sciences research involving one or more of the DURC Agents, the agency originally providing the funding shall be considered the US Funding Agency.

III. REQUIREMENTS FOR PRINCIPAL INVESTIGATORS

A Principal Investigator ("PI") must submit for institutional review any anticipated or proposed research that meets any of the following criteria:

- the research directly involves nonattenuated forms of one or more of the DURC Agents;
- the research with nonattenuated forms of one of more of the DURC Agents produces, aims to produce or can reasonably be anticipated to produce one or more Experimental Effects of Concern; or
- the PI concludes that his/her research may meet the definition of DURC.

If a PI's research meets any of the foregoing criteria, he/she will promptly notify the IRE by contacting EHS with the relevant information, including the PI's assessment as to whether the research produces, aims to produce or is reasonably anticipated to produce one of more of the Experimental Effects of Concern and provide documentation indicating the reasons for his/her conclusion. EHS will provide a form for this notification. ***The research may not proceed without IRE approval, either through a determination that it is not DURC subject to this Policy, or because an approved mitigation plan has been implemented.***

Yale's process for identifying possible DURC also includes

- queries on EHS/BSC forms for reviewing research EHS, including DURC questions on the Yale IBC Registration of Recombinant DNA Research, the Yale EHS Request to Use Infections Agents Registration and the Yale EHS FORM 01 Biological Materials Registration.
- the restricted items list though SciQuest, which triggers for DURC agents.
- EHS Safety Advisors, who check for new pathogen and high risk toxin research at least annually during their biosafety inspections

IV. POLICY REQUIREMENTS FOR INSTITUTIONAL REVIEW

The 2014 Policy requires an institution to designate an IRE to execute the institutional review of potential DURC Research. The Yale IRE is made up of the members of the Yale BSC. On a case-by-case basis, the IRE will recuse any member who is involved in the research project in question or has a direct financial interest, except to provide specific information requested by the IRE.

A. Review by the IRE for DURC Agents and Experimental Effects

The first step of the IRE review process is to verify that the subject research directly involves nonattenuated forms of one or more of the DURC Agents based on the materials provided by the PI and any other relevant materials. The USG also deems any of the following need not be reviewed under the 2014 Policy:

- The use of any of the DURC Agents in attenuated forms (unless the experiment will reconstitute a virulent agent);
- The use of the genes from any of the DURC Agents;
- *In silico* experiments (e.g., modeling experiments, bioinformatics approaches) involving the biology of the DURC Agents); or
- Research relating to the public, animal and agricultural health impact of any of the DURC Agents (e.g., modeling the effects of a toxin, developing new methods to deliver a vaccine, developing surveillance mechanisms for a DURC Agent).

The IRE will notify the PI in writing that the research may proceed, provided all other requirements are met, if it concludes that the research does not involve one or more DURC Agents or is not intended for review under the above criteria.

If the IRE concludes that the research does involve one or more DURC Agents, it will assess whether the research produces, aims to produce or is reasonably anticipated to produce one of more of the Experimental Effects of Concern based on review of the assessment and materials provided by the PI and any other relevant materials.

Based on the foregoing, if the IRE concludes that the research does not involve Experimental Effects of Concern and is therefore not subject to additional DURC oversight, it will notify the PI in writing that the research may proceed if all other requirements are met.

B. Review for DURC

If the IRE concludes that the research does involve one or more DURC Agents and Experimental Effects of Concern, the IRE will assess the risks of the research and determine whether the research is DURC. In so doing, it should examine descriptions of the research, the PI's assessments and other relevant information such as the project proposal, any project reports, any previous outcomes of Dual Use reviews and examples of similar research in the literature.

Guidance on points to consider while making this assessment can be found in Section C.2 of the Companion Guide1. The applicable US Funding Agency may be consulted for advice.

If the IRE determines that the subject research does not meet the definition of DURC, it is not subject to additional institutional oversight and the IRE will promptly so notify the PI and, within 30 days, the applicable US Funding Agency. The IRE will inform the PI that the research may proceed if other requirements are met. The IRE and/or the ICDUR may consult with the US Funding Agency with respect to the Committee's determination.

C. Development of Mitigation Plan

If the IRE concludes that the subject research does meet the definition of DURC, it will promptly so notify the PI and within 30 calendar days, the applicable US Funding Agency, and shall proceed to develop a risk mitigation plan. The IRE will inform the PI that the research may not proceed without implementation of the risk mitigation plan as approved by the IRE.

In order to determine the acceptable level of risk associated with the DURC and the best mitigation strategies, the IRE will assess the potential benefits of the Research, with input from the PI, and then weigh the risks and benefits. Guidance on points to consider in making this assessment can be found in Section C.2 of the Companion Guide.

The IRE will then develop a draft risk mitigation plan (the "Risk Mitigation Plan") in consultation with the PI. The Plan should indicate the DURC associated risks, the specific risk mitigation measure to be employed and how these measure address the identified risks. Strategies for mitigating risks include:

- Applying additional biosafety or biosecurity measures
- Modifying the experimental design or methodology
- Planning for medical countermeasures

[1] Tools for the Identification, Assessment, Management, and Responsible Communication of Dual Use Research of Concern (the "Companion Guide") https://www.phe.gov/s3/dualuse/Documents/durc-companion-guide.pdf.

- Determining a plan for responsibly communicating the research findings
- Educating and training research staff
- Developing a specific monitoring plan
- Not conducting certain aspects of the research.

Guidance on points to consider in drafting a Risk Mitigation Plan and in creating a responsible communication plan can be found in Sections D and F of the Companion Guide. The applicable US Funding Agency may also be consulted for advice.

At the conclusion of its review, the IRE will submit its findings and its recommendations as to the elements of the draft Risk Mitigation Plan to the Office of the Provost. The

Deputy Provost, or his designee, will decide whether or not to act on the recommendations of the IRE as to whether the research constitutes DURC and the adequacy of the IRE's draft Risk Mitigation Plan and may require revisions to the draft Plan.

The Deputy Provost's decision and any other institutional decision regarding DURC may be appealed by the affected PI to the Provost. The Provost will have the final word as to all institutional decisions regarding DURC that have been appealed.

D. Notification to the US Funding Agency and Finalization of the Risk Mitigation Plan

Within 90 calendar days following the final institutional approval of the draft Risk Mitigation Plan by the cognizant Deputy Provost (or the Provost), the ICDUR shall submit such draft Plan to the applicable USG Funding Agency for final review and approval. The USG Funding Agency must provide an initial response within 30 calendar days following receipt of the draft Plan. The ICDUR and the PI will work with the USG Funding Agency to respond to any questions or concerns it may have regarding the draft Risk Mitigation Plan. The USG Funding Agency must finalize the Plan within 60 days following receipt of the draft Plan. The cognizant Deputy Provost must also approve the final Risk Mitigation Plan. Upon approval by both the USG Funding Agency and the Deputy Provost, the IRE will notify the PI that the research may proceed according to the Risk Mitigation Plan, provided other requirements are met.

E. Sub-awards

If elements of a potential DURC Research project are being carried out at multiple institutions through a subaward with a primary institution that directly receives the grant or contract from the US Funding Agency (the "Prime Institution"), the Prime Institution will be responsible for notifying the applicable US Funding Agency of research that may constitute DURC and if such research is determined to be DURC, providing copies of each institution's Risk Mitigation Plan. The Prime Institution should also ensure that DURC oversight is consistently applied by all entities participating in the collaboration.
If Yale is not the Prime Institution, and the Prime Institution's procedures or standards are less rigorous than Yale's, Yale reserves the right to apply more rigorous procedures or standards.

V. ONGOING INSTITUTIONAL RESPONSIBILITIES

1. Responsibilities of the PI

The PI will:

- Conduct DURC Research in accordance with the final Risk Mitigation Plan;
- Notify the ICDUR of the addition of any DURC Agents or Experimental Effects of Concern, or any other substantive change in the conduct of the DURC Research;
- Notify EHS if for whatever reason (e.g., changes in the research, new discoveries), he/she feels that the research should be reconsidered by the IRE because it might constitute DURC, or is no longer DURC; and
- Ensure that laboratory personnel (i.e., those under the supervision of laboratory leadership, including graduate students, postdoctoral fellows, research technicians, laboratory staff and visiting scientists) conducting research with one of more of DURC Agents have received EHS-approved education and training on DURC.

2. Responsibilities of the ICDUR

The ICDUR shall:

- Ensure that the IRE reviews each DURC Research Risk Mitigation Plan annually;
- Provide education and training on DURC for individuals conducting research with one or more of the DURC Agents and maintain records of such education and training for the term of the research grant or contract plus three years after its completion;
- Maintain records of institutional DURC reviews and completed Risk Mitigation Plans for no less than eight years, unless a shorter period is required by law or regulation;
- Notify the applicable US Funding Agency within 30 calendar days of any change in the status of any DURC, including whether such research has been determined by the IRE to no longer meet the definition of DURC. The notification should include details of any changes to an approved Risk Mitigation Plan, which must be approved by the US Funding Agency; and
- Report within 30 calendar days to the applicable US Funding Agency instances of noncompliance with this Policy, as well as mitigation measures undertaken by the University to prevent recurrences of similar noncompliance.

3. Responsibilities of the IRE

The IRE shall review, at least annually, all active Risk Mitigation Plans at the University. In reviewing such Plans, the IRE will follow the same procedures as are described in this Policy. Guidance on points to consider while conducting this review may be found in the Companion Guide, Section E. If the research in question still constitutes DURC, the IRE, working with the PI, should modify the applicable Risk Mitigation Plan as needed to ensure that the Plan still adequately mitigates the risks associated with the DURC.

Appendix E Registration and Approval of rDNA Experiments - Poster

Registration and Approval of rDNA Experiments

RECOMBINANT and SYNTHETIC NUCLEIC ACIDS

YALE BIOLOGICAL SAFETY COMMITTEE **April 2019 (rev.)**

This outline provides an overview of the "Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules" (NIH Guidelines). It is the responsibility of each investigator to make sure that their laboratory is in compliance with these Guidelines. If your experiments require registration, check the NIH Guidelines for the relevant regulatory section and the appropriate biosafety level or contact the Biosafety Office or your Safety Advisor for assistance. For copies of the NIH Guidelines or rDNA registration forms, please call Environmental Health & Safety (EHS) at 785-3550.

OEHS contacts: Phone: (203) 785-3550 Fax: 785-7588 Website: https://ehs.yale.edu/

Yale rDNA Forms and Information Regarding rDNA: https://ehs.yale.edu/recombinant-dna

NIH Office of Science Policy website: https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/

Experiments which must be registered and approved prior to initiation:	Examples:
1. Deliberate transfer of a drug resistance trait to a microorganism (if it could compromise the use of the drug to control disease agents in human, animals, or agriculture);	1. Transferring a drug resistance trait that is used, had previously been used, may be used (outside the U.S.), or that is related to other drugs that are used to treat or control disease agents. Examples include: Transfer of Erythromycin resistance into Borrelia burgdorferi; Transfer of Pyrimethamine resistance into Toxoplasma gondii; Transfer of Chloramphenicol resistance into Rickettsia conorii; Transfer of Tetracycline resistance into Porphyromonas gingivalis.
2. Human gene transfer experiments;	2. Use of a defective adenoviral vector to deliver the CFTR gene intranasally to patients with Cystic Fibrosis; Introduction of a HSV-TK transduced cell line into patients with epithelial ovarian carcinoma, followed by therapy with Gancyclovir.
3. Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of < 100 µg/kg body weight;	3. Cloning toxins (or using plasmids that express toxins with low LD50's) such as Botulinum, Tetrodotoxin, Ricin, T-2, Saxitoxin, Abrin, Tetanus, Shigella Dysenteriae, Pertussis, Staph Aureus Beta, Shiga Toxin, and Conotoxins;
4. Experiments using human or animal pathogens as host-vector systems;	4. Use of pathogens or defective pathogen vectors (with or without helper virus), such as Adenovirus, Adeno-Associated virus, Baculovirus, Herpes virus, Lentivirus, Retrovirus, Vaccinia and Vesicular Stomatitis Virus.
5. Cloning of DNA or RNA from all Risk Group 3, 4, or restricted pathogens (includes HIV and human tumor viruses), as well as Risk Group 2 experiments involving ≥ 50 % of genetic material;	5. rDNA experiments involving any quantity of genetic material from a Risk Group 3 or higher pathogens (e.g., HIV, HTLV-1 & II, Prions, Mycobacterium tuberculosis, West Nile Virus, Lymphocytic Choriomeningitis Virus, and Rickettsia typhi. Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.
6. Recombinant DNA experiments involving whole animals or plants:	6. Creation of transgenic animals or plants (mice, rats, zebra fish, drosophila, C. elegans etc.), or knockout animals that leave genetic material in the animal as part of the silencing of the gene. Note: the purchase (or transfer to your lab) of previously created transgenic rodents is exempt from the regulations.
7. Large-scale DNA work (i.e. ≥ 10 liters of culture combined).	7. Use of a 10 L fermenter or growing up five 2 L flasks of rDNA culture (i.e. E. coli K-12) qualifies as a large-scale experiment at Yale University.

NIH Guidelines Definitions and Information on

Recombinant or Synthetic Nucleic Acids

Section I-B. Definition of Recombinant and Synthetic Nucleic Acid Molecules
In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:
(i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;
(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
(iii) molecules that result from the replication of those described in (i) or (ii) above.

Section III-C-1. Human gene transfer is the deliberate transfer into human research participants of either:
1.Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acidmolecules, or
2.Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acidmolecules, that meet any one of the following criteria:a.Contain more than 100 nucleotides; or
b.Possess biological properties that enable integration into the genome (e.g., *cis*elements involved in integration); or
c.Have the potential to replicate in a cell; or
d.Can be translated or transcribed.

Synthetic Nucleic Acid Experiments that are covered by the Guidelines:

- Research that presents biosafety risks equivalent to rDNA research that is subject to the NIH Guidelines such as research with a genetically modified virus or a vector derived solely by synthetic techniques. Research involving synthetic nucleic acid molecules will require registration if:
    - The molecules can replicate
    - They can generate nucleic acids that can replicate in a living cell
    - They can integrate into a host cell's DNA
    - They produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms/kilogram body weight
    - They synthesize an organism that doesn't occur naturally outside of a laboratory setting (i.e. 1918 H1N1 Influenza)
- Human gene transfer experiments or clinical protocols with synthetic nucleic acid molecules if any of the following criteria are met - the synthetic nucleic acid molecules:
    - Contains more than 100 nucleotides; or
    - Possess biological properties that enable integration into the genome (e.g. cis elements involved in integration); or
    - Have the potential to replicate in a cell; or
    - Can be translated or transcribed.

Synthetic Nucleic Acid Experiments that are EXEMPT from the Guidelines:

- Introduction of certain synthetic nucleic acids into a biological system that is not expected to present a biosafety risk that requires review by the IBC
- Introduction of synthetic nucleic acid molecules into biological systems akin to processes of nucleic acid transfer that already occur in nature.
- Experiments with synthetic nucleic acid molecules that are not contained in cells, organisms or viruses
- Those synthetic nucleic acid molecules that meet the following criteria shall be exempt:
    4) Those that can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g. oligonucleotides or other synthetic that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and
    5) Those that are not designed to integrate into DNA, and

Those that do not produce a toxin that is lethal for vertebrates at and LD50 of less than 100 nanograms per kilogram body weight

Appendix F Human Gene Transfer Clinical Trials

The following guide has been prepared to assist Principal Investigators and their supporting groups with the registration and review process for clinical research studies that involve the use of recombinant or synthetic nucleic acid molecules in human subjects. Although the guide provides information about the submission and registration process and ongoing requirements after initiation of a human gene transfer protocol at Yale, it may not address every question that may arise. If you have any questions, please call the EHS Office at (203) 785-3550 and ask to speak to the Biosafety Office or the Safety Advisor assigned to your research or clinical area.

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Introduction

Proposed clinical trials involving human gene transfer (HGT) require registration with, and approval from, both campus and federal agencies before initiation. HGT is the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects. The National Institutes of Health (NIH) formal definition of HGT is provided in the next paragraph. This document outlines the Yale University Biological Safety Committee (Committee) requirements for HGT protocols. Additional federal requirements (NIH and United States Food and Drug Administration) for these experiments are described in Section III-C of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines), April 2019, and in the Code of Federal Regulations, 21 CFR, Part 312 (FDA Points to Consider).

NIH Definition of Human Gene Transfer Research (HGT) and Exemptions

NIH Definition of Human Gene Transfer Research (HGT)

Section III–C–1. Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants.

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
2. Synthetic nucleic acid molecules, or
3. DNA or RNA derived from synthetic nucleic acid molecules that meet any one of the following criteria:
    a. Contain more than 100 nucleotides; or
    b. Possess biological properties that enable integration into the genome (*e.g., cis* elements involved in integration); or
    c. Have the potential to replicate in a cell; or
    d. Can be translated or transcribed

Expanded Access Exemption

The deliberate transfer of recombinant or synthetic nucleic acids into one human research participant, conducted under a Food and Drug Administration (FDA) regulated individual patient expanded access Investigational New Drug (IND) or protocol, including for emergency use, is not research subject to the NIH Guidelines and thus does not need to be submitted to an IBC for review and approval. These protocols still require registration with the Yale Human Investigation Committee.

Treatment Exemption

If the use of recombinant or synthetic nucleic acid molecules is for "treatment", such as used in an emergency IND, review by the Yale Biological Safety Committee would not be required. Only protocols involving recombinant or synthetic nucleic acid molecules in human subjects classified as "research" require Committee review and authorization prior to initiation. These protocols still require registration with the Yale Human Investigation Committee.

On Campus Registrations and Approvals

It is recommended that HGT registrations or notifications are pursued in the following order:

A. Notification to Yale New Haven Hospital (YNHH) Hospital Epidemiology and YNHH Occupational Health of the request to conduct a HGT protocol at the YNHH.

   It is preferred that the Principal Investigator invite the Sponsor to YNHH to provide a presentation on the description of the proposed project for representatives of these two groups AND representatives from all groups who may participate in the project, especially if any hazards are involved. This would include the pharmacists who will handle and prepare the study drug for administration, the physicians and nurses who will have to deliver the study drug, and any other healthcare workers who will work with study subjects.

   i. It is imperative that the Principal Investigator or Department confirm that there is a sufficient number of personnel from the groups above (pharmacists, nurses, doctors, and other health care providers) willing to participate in the protocol prior to registration with the following campus groups.

   ii. Verification that personnel will participate on the project is required prior to the initiation of review of the protocol by the Yale Biological Safety Committee.

B. Registration with the Yale Biological Safety Committee and the Committee's Human Gene Transfer Subcommittee

General registrations must be received by the Biological Safety Committee by the 1st Thursday of each month, to have an opportunity to be reviewed by the Committee at the monthly meeting on the 3rd Thursday of each month.

As these protocols are usually more complex than a standard recombinant DNA project that does not involve human subjects, the "best" day to submit a Human Gene Transfer Protocol is on the 3rd Thursday of the month. This will guarantee the "longest" time in between monthly Committee meetings to allow sufficient time for review.

C. Registration with the Yale Human Research Protection Program Human Investigation Committee

Contact information for each of these groups is provided below.

Yale New Haven Hospital

YNHH Hospital Epidemiology (203) 688-4634
YNHH Occupational Health – York St (203) 688-4242

Note: Human Gene Transfer (HGT) at Yale New Haven Hospital

To determine if your proposed HGT research may require review and clearance from the YNHH Hospital Epidemiologist or Infection Control Department, please contact them at (203) 688-4634. All personnel who handle potential hazards at YNHH must notify the YNHH Occupational Health Office at (203) 688-4242 to receive additional health and safety information related to the project. This includes physicians, nurses, pharmacists, and others who may handle the study drug or study subjects when potential hazards are involved. **Notifications should be made well in advance of the proposed start date to initiate review of any HGT experiments planned within YNHH.**

Once your HGT Protocol is approved by the

Yale HIC and the Yale IBC:

The Yale IBC approval letter will require a "start-up meeting" with YNHH. It is advisable that you contact YNHH immediately after receiving all approvals to set up this meeting.

Please don't hesitate to contact Biosafety at (203) 785-3550 if you have any questions.

Yale Biological Safety Committee (IBC)

(203) 785-3550 (through Biosafety Representatives)

http://ehs.yale.edu/biosafety-committee

http://provost.yale.edu/committees

Yale Human Research Protection Program (IRB): Human Investigation Committee

(203) 785-4688

http://www.yale.edu/hrpp/

http://www.yale.edu/hrpp/forms-templates/biomedical.html

HRPP Contact Information:

25 Science Park, 3rd Floor
150 Munson Street
PO Box 208327
New Haven, CT 06520-8327
Phone: (203) 785-4688
Fax: (203) 785-2847
hrpp@yale.edu

Yale University Human Research Protection Program (HRPP):

The Yale HRPP must approve all experiments involving human subjects prior to initiation. Please contact the HRPP at (203) 785-4688 for information on their requirements.

Federal Registration and Approval

As of August 2018, registration with the NIH for recombinant and synthetic nucleic acids research involving human subjects is no longer required. However, projects with recombinant and synthetic nucleic acids research involving human subjects must be registered with and approved by the FDA.

FDA Center for Biologics Evaluation and Research:
http://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/default.htm

21 CFR Part 312:
http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&SID=528ebe054b8cf1dc958289ee9fc1f972&rgn=div5&view=text&node=21:5.0.1.1.3&idno=21

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 (HGT) Clinical Trials at Yale University

To initiate the review of a proposed HGT clinical trial, please follow the instructions on pages 9 – 12 of this document. Please forward your documents to the Yale Biological Safety Committee at the address below. You may also submit your required documents electronically to ehs@yale.edu.

The Yale Biological Safety Committee meets on the 3rd Thursday of each month.

The deadline for the receipt of recombinant DNA and HGT protocols is the 1st Thursday of each month.

As HGT protocols are usually more complex than non-clinical recombinant DNA protocols, it is highly recommended that they are submitted well before the Committee meeting date. **THE IDEAL DATE FOR THE SUBMISSION OF AN HGT PROTOCOL IS THE 3RD THURSDAY OF THE MONTH. This will provide at least 30 days for the review of the protocol by the Human Gene Transfer Subcommittee for the Yale Biological Safety Committee.**

The latest edition of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines) was published in April 2019. To obtain a copy of the NIH Guidelines, access the NIH Office of Science Policy (OSP) web site at the URL shown below or contact the Biosafety Office at (203)785-3550.

The Yale Biological Safety Committee
C/O Biosafety Office
Yale Office of Environmental Health & Safety
135 College Street, Suite 100
New Haven, CT 06510

Contact person: Biosafety Officer, (203) 785-3550

NIH OSP web site:
https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/

Yale Biological Safety Committee Submission Requirements for the Review of Human Gene Transfer (HGT) Protocols

For initial review by the HGT Subcommittee of the Yale Biological Safety Committee the following are required:

- Yale EHS IBC HGT Registration Form ('Protocol Profile') [included on pages 9 - 12]
- Sponsor's Protocol (or Clinical Study Protocol)
- Investigator's Brochure (or Principal Investigator's Brochure)

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

- The Yale Biological Safety Committee HGT Registration Form ('Protocol Profile')
- Scientific abstract (as provided in the (Principal) Investigator's brochure)
- Non-technical abstract (as provided in the informed consent document)
- A copy of the HIC Informed Consent Document (a draft is acceptable for Yale Biological Safety Committee review process)
- Sponsor's Protocol (Clinical Study Protocol)
- Principal Investigator's (Investigator's) Brochure
- Sponsor's Pharmacy Manual (and if available Sponsor's Nursing Manual)
- Curricula vitae (2 pages) for each key professional in biographical sketch format (The Principal Investigator and 2 to 3 other lead personnel is sufficient)
- The proposed location for vector production and description of the Good Manufacturing or Good Clinical Practices that will be utilized to prepare the research material (study drug)
- A copy of the Certificate of Analysis (CoA) for sterility for each lot of study drug made at Yale or sent to the University for this experiment (this may be submitted after Yale Biological Safety Committee review but will be needed prior to administration of the study drug to the first patient).

Yale's Institutional Biological Safety Committee (IBC) Submission Requirements for the Review of Human Gene Transfer (HGT) Protocols Checklist

1. Documents required to initiate review process with the IBC

Check	Document
☐	Yale EHS IBC HGT Registration Form ('Protocol Profile') [*included on pages 9 - 12]*
☐	Sponsor's Protocol (or Clinical Study Protocol)
☐	Investigator's Brochure (or Principal Investigator's Brochure)

2. Documents that are required prior to initiation of the protocol

Check	Document
☐	Scientific Abstract [*as provided in (Principal) Investigator's Brochure*]
☐	Non-technical Abstract [*as provided in the Informed Consent Document*]
☐	Curricula vitae (e. g. 2 pages) for the Principal Investigator and 1 or 2 others `in biographical sketch format
☐	Copy of the HIC Informed Consent Document *(draft is acceptable)*
☐	Sponsor's Pharmacy Manual
☐	Sponsor's Nursing Manual *(if available)*
☐	Yale IRES-IRB Application to Involve Human Subjects in Biomedical Research *(PDF of pre-submission is acceptable)*
☐	Proposed location for production of the study drug and/or vector and a description of the Good Manufacturing or Good Clinical Practices that will be utilized to prepare the study drug or vector (*if applicable)*
☐	Copy of the Certificate of Analysis (CoA) for sterility for each lot of the study drug or vector made at Yale or sent to the University for this experiment (*if applicable*)

Yale EHS IBC Human Gene Transfer (HGT) Registration Form (‘Protocol Profile’)

If this information is already detailed in one of your existing documents, please provide the document title and page number(s) in the applicable spaces below

Principal Investigator	Sponsor: Manufacturer (of product):
Title of Protocol	
FDA IND #: Sponsor Protocol #: Yale HIC#:	
Targeted Disease or Clinical Aim of Project:	
Summary of preclinical studies conducted in support of the proposed clinical trial or reference to the specific section of the protocol providing this information.	
Product description	
Derivation of the delivery vector system including the source (e.g., viral, bacterial, plasmid), associated modifications (i.e., deletions to attenuate or self-inactivate, encapsulation in any synthetic complex, changes to tropisms), and previous clinical experience with the system	

Genetic content of the transgene or nucleic acid delivered, including the species source of the sequence, and whether any modifications have been made (e.g., mutations, deletions, truncations)
Any other material to be used in preparation of the agent (vector and transgene) to be administered to research participants (e.g., helper virus, packaging cell line, carrier particles)?
Intended *ex vivo* or *in vivo* target cells and transduction efficiency
Gene transfer agent delivery method
Methods for replication-competent virus testing Certificate of Analysis for adventitious agents and replication competency (if applicable) must be provided for each lot of study drug used at Yale.
Other Clinical Trial Sites approved for the project: Other sites proposed for the study? Total number of subjects enrolled to date:

Please outline the procedures that will be followed in the event of a spill of the investigational agent: Please outline the procedures that will be followed in the event of a staff member's exposure to the investigational agent:
What supporting safety data (cell culture, animal model, etc.) was utilized to move forward with research involving human subjects? Please provide safety data for the recombinant molecule and other study drugs involved.
Describe the dosing regimen for the recombinant molecule and other study drugs (please include starting dose, maximum allowable dose, and the study administration schedule).
How many cycles are allowed for study subjects:
Provide any history of use of the recombinant molecule in other studies (include the total number of subjects, and numbers of Adverse Events and Serious Adverse Events)

Please provide the proposed study location (where the drug will be administered)? Has the Principal Investigator confirmed participation by a suitable number of healthcare workers to participate in all required aspects of the study?
Will the YNHH Pharmacy been involved? Have Pharmacy personnel confirmed their participation in the study?
Please provide the date the study presentation was provided by the sponsor to the potential study participants at YNHH:

Note: Any application submitted shall not contain any document that is designated as 'confidential' in its entirety. If a determination has been made that a specific portion of a document should be considered proprietary or trade secret, each specific portion shall be clearly identified as such. If a specific portion of the submission is identified to be proprietary or trade secret, the submission to the Yale IBC must contain a letter that: (1) clearly indicates what select portions of the application contain information considered as proprietary or trade secret, and (2) provides justification as to why this information is proprietary or trade secret. The justification must be able to demonstrate with specificity how release of that information will reveal a trade secret or will result in substantial competitive harm.

Pathway for human gene transfer (HGT) protocols at Yale University

1. If the protocol will be conducted at YNHH and involves hazards, the Principal Investigator must notify YNHH Hospital Epidemiology/Infection Prevention and YNHH Occupational Health for additional review and information.
2. The Sponsor conducts an introductory meeting with the Principal Investigator and all possible healthcare workers, including pharmacy staff, who may participate in the project. The presentation covers the rational for the project and any hazards involved for the subjects and healthcare workers.
3. The Principal Investigator verifies that there is sufficient staffing after the Sponsor's presentation to participate in all required aspects of the study.
4. Principal Investigator submits the required documentation listed above (pages 7 - 12) to the Yale Biological Safety Committee.
5. The EHS Biosafety Office verifies that all documentation has been received and submits the complete protocol to members of the Yale Biological Safety Committee's HGT Subcommittee.
6. If needed, an HGT Subcommittee meeting is scheduled with the Principal Investigator and technical representatives from the Sponsor who are familiar with the recombinant molecules utilized in the protocol. Sponsor representatives generally participate by teleconference at the live HGT meeting. HGT Subcommittee meetings are scheduled as needed and meeting dates are coordinated with HGT Subcommittee members and representatives from the Principal Investigator's research team.
7. The chair of the HGT Subcommittee, or designee, will provide the recommendation of the HGT Subcommittee on the protocol to the Yale Biological Safety Committee.
8. The Yale Biological Safety Committee will review the recommendations from the HGT Subcommittee and will vote on the protocol. Yale Biological Safety Committee meetings are usually held monthly on the third Thursday of the month.
9. The results of the Yale Biological Safety Committee review are communicated to the Principal Investigator and the Yale Human Investigation Committee. If approved, the Principal Investigator will receive an approval letter for the protocol from the Yale Biological Safety Committee.
10. Principal Investigator submits a registration for conducting research involving human subjects to the Yale Human Investigation Committee.
11. The Yale Human Investigation Committee will complete its review of the project. If approved, the Principal Investigator will receive an approval letter from the Human Investigation Committee.
12. For protocols being conducted at the Yale New Haven Hospital: Once the Principal Investigator receives approvals from both Committees (the Biological Safety Committee and the Human Investigation Committee), the Principal investigator must contact the Yale New Haven Hospital Infection Control Committee to set up a protocol review meeting prior to the initiation of the protocol.

Adverse Events and Reporting Requirements

Unintended Consequences Among Staff

If a clinical study team member experiences an exposure, injury, or infection related to their participation in the study, this must be reported to both the Yale Biological Safety Committee and the Yale Human Investigation Committee. Each Committee has subsequent reporting requirements to their respective federal agencies (NIH Office of Science Policy and the FDA).

Adverse Events Experienced by Study Subjects

All adverse events must be reported in an annual data summary that is prepared for the Yale HIC, the Yale Biological Safety Committee, the FDA, and the sponsor. Any **Serious Adverse Events (SAE's)** must be reported by telephone (to who?) 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, and the FDA 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 number, 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.

April 2019 Yale and NIH Points to Consider for the IBC Review of Human Gene Transfer Protocols

The following documentation must be submitted to the Yale Institutional Biological Safety Committee to initiate the review of an HGT protocol.

- A scientific abstract as provided in (Principal) Investigator's Brochure
- The proposed clinical protocol, including tables, figures, and any relevant publications

Ensure that the information described in the bulleted list below is included in the materials submitted to the Committee. The information may be described in the Clinical Study Protocol or the Investigator's Brochure. If not, please provide the information in a separate document.

- Summary of preclinical studies conducted in support of the proposed clinical trial or reference to the specific section of the protocol providing this information.
- Product description, for instance:
    - Derivation of the delivery vector system including the source (e.g., viral, bacterial, plasmid), associated modifications (i.e., deletions to attenuate or self-inactivate, encapsulation in any synthetic complex, changes to tropisms), and previous clinical experience with the system
    - Genetic content of the transgene or nucleic acid delivered, including the species source of the sequence, and whether any modifications have been made (e.g., mutations, deletions, truncations)
    - Any other material to be used in preparation of the agent (vector and transgene) to be administered to research participants (e.g., helper virus, packaging cell line, carrier particles)
    - Methods for replication-competent virus testing
    - Intended *ex vivo* or *in vivo* target cells and transduction efficiency
    - Gene transfer agent delivery method

Note: Any application submitted shall not contain any document that is designated as 'confidential' in its entirety. If a determination has been made that a specific portion of a document should be considered proprietary or trade secret, each specific portion shall be clearly identified as such. If a specific portion of the submission is identified to be proprietary or trade secret, the submission to the Yale IBC must contain a letter that: (1) clearly indicates what select portions of the application contain information considered as proprietary or trade secret, and (2) provides justification as to why this information is proprietary or trade secret. The justification must be able to demonstrate with specificity how release of that information will reveal a trade secret or will result in substantial competitive harm.

April 2019 Amendment of the *NIH Guidelines* - NIH Office of Science Policy

This section provides many of the frequently asked questions (FAQs) related to human gene transfer from the NIH Office of Science Policy web site. Additional FAQs are available on the NIH Office of Science Policy web site at https://osp.od.nih.gov/biotechnology/faqs-on-the-nih-guidelines-research-synthetic-nucleic-acid-molecules/.

What specific changes have been made to the *NIH Guidelines* regarding human gene transfer (HGT) protocol submission and reporting requirements to NIH's Office of Science Policy (OSP)?

Under the *NIH Guidelines,* individual HGT protocol submission and reporting to NIH/OSP are no longer required. Specifically, NIH/OSP will not: accept or register new HGT protocols; convene the Recombinant DNA Advisory Committee (RAC) to review individual HGT protocols; accept annual reports, safety reports, amendments or other documentation for any HGT protocols previously registered under the *NIH Guidelines* (formerly, Appendix MI-C).

What changes have been made to the *NIH Guidelines* regarding the roles and responsibilities of relevant entities?

It is important to note that while NIH is streamlining individual human gene transfer (HGT) protocol reporting requirements, robust oversight over HGT research will continue through both Federal and local oversight bodies. The roles and responsibilities of investigators, institutions, and oversight bodies involved in HGT research remain the same, except: The roles of Institutional Biosafety Committees in reviewing HGT research have been modified to be consistent with the review of other research covered by the *NIH Guidelines*.

Principal Investigators (PIs) will no longer be responsible for ensuring requirements for protocol submission, review, and reporting for HGT protocols to NIH's Office of Science Policy are addressed, since these responsibilities have been eliminated. All other roles and responsibilities for PIs will remain the same.

Because the Novel and Exceptional Technology and Research Advisory Committee (NExTRAC) will now focus on advising the NIH Director on scientific, safety, and ethical issues associated with emerging biotechnologies (which is not necessarily limited to recombinant or synthetic nucleic acid molecule research), non-historical references to the Recombinant DNA Advisory Committee will be removed from the *NIH Guidelines*. The roles and responsibilities will be delineated in the charter, as is typical for such committees.

Is human gene transfer (HGT) research still covered under the *NIH Guidelines*? What is required before a Principal Investigator or sponsor can initiate HGT research and begin enrollment/recruitment/accrual?

Yes. When conducted by an entity subject to the *NIH Guidelines* (see Section I-C), HGT research (see Section III-C) is still covered, as protocols must still be reviewed and approved by Institutional Biosafety Committees to assess biosafety considerations at the clinical trial site. In addition, all other applicable institutional and regulatory authorization(s) and approvals must be obtained before any research with human participants can be initiated.

If human gene transfer (HGT) protocols are no longer registered with NIH's Office of Science Policy (OSP), will sites conducting only HGT research still need to register their Institutional Biosafety Committees (IBCs) with NIH/OSP?

Yes. All entities conducting research subject to the *NIH Guidelines*, including HGT research, must have an appropriately constituted IBC registered with NIH/OSP. For additional information on registering an IBC, see Section IV-B-2 of the *NIH Guidelines* and on the NIH/OSP website: https://osp.od.nih.gov/biotechnology/faqs-on-ibc-administration/.

Is human gene transfer research conducted under Food and Drug Administration (FDA)-regulated individual patient expanded access Investigational New Drug (IND) applications subject to the *NIH Guidelines*?

No. The deliberate transfer of recombinant or synthetic nucleic acids into one human research participant, conducted under an FDA-regulated individual patient expanded access IND or protocol, including for emergency use, is not research subject to the *NIH Guidelines* and thus, does not need to be submitted to an Institutional Biosafety Committee for review and approval. Specific guidance regarding FDA requirements is provided at:

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expanded-access-investigational-drugs-treatment-use-questions-and-answers

Will Institutional Biosafety Committees (IBCs) be required to change their review processes? What aspects of human gene transfer (HGT) research should IBCs focus on?

The focus of the IBC review of HGT research should be equivalent to their review of the biosafety aspects of other covered research, e.g.:

- required containment levels
- potential for shedding
- safety and training of laboratory/technical personnel involved in the clinical protocol details of the facilities
- adequacy and maintenance of safety equipment that may be used in support of the clinical protocol
- safety procedures and practices when working with the product and during administration to a protocol participant
- reporting of biosafety accidents and incidents occurring during conduct of the protocol
- approving emergency response plans for accidental spills and personnel contamination

As with other research reviewed by IBCs, IBCs should determine what information they require to complete their biosafety review of HGT protocols.

IBC oversight may conclude after the last participant is administered the final dose of product. However, IBCs may choose to establish other end points for oversight, based on their biosafety assessment of the proposed research.

Other aspects of HGT research, such as review of informed consent, are under the purview of the Food and Drug Administration and Institutional Review Boards.

Should biosafety incidents occurring during the conduct of human gene transfer (HGT) research still be reported to NIH's Office of Science Policy (OSP)?

Yes. The *NIH Guidelines* require that "…any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" be reported to NIH. Reports of incidents can be emailed to NIHGuidelines@od.nih.gov. Relevant incidents would include spills and accidents that result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules in the laboratory, rather than serious adverse events that may occur in the conduct of HGT research. Additional information on incident reporting and a reporting template are available on the NIH/OSP website at https://osp.od.nih.gov/biotechnology/nih-guidelines/

March 2013 Changes to the NIH Guidelines (Addressing Synthetic Biology)

In March 2013, the NIH Guidelines will have a new title, the "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules". Even with a new title, the document is still referenced as the "NIH Guidelines." The change was made to keep pace with rapid technological advancements in synthetic biology.

The NIH Guidelines were expanded to include new language to address nucleic acid molecules created solely by synthetic means, and will include:

- Recombinant nucleic acid molecules;
- Synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules; and
- Cells, organisms, and viruses containing such molecules.

The phrase "recombinant or synthetic nucleic acid molecules" replaced the term "recombinant DNA molecules" throughout the text of the NIH Guidelines.

Updated definition for recombinant and synthetic nucleic acid molecules:

(i) Molecules that a) are constructed by joining nucleic acid molecules and b) can replicate in a living cell (i.e. recombinant nucleic acids);
(ii) Nucleic acid molecules that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e. synthetic nucleic acids); or
(iii) Molecules that result from the replication of those described in (i) or (ii) above.

HGT Synthetic Nucleic Acid Experiments that are covered by the Guidelines:

Human gene transfer (HGT) experiments with synthetic nucleic acid molecules if any of the following criteria are met: The synthetic nucleic acid molecules:

- Contains more than 100 nucleotides; or
- Possess biological properties that enable integration into the genome (e.g. cis elements involved in integration); or
- Have the potential to replicate in a cell; or
- Can be translated or transcribed.

Standard Approval Letter Language from the Yale Biological Safety Committee for Human Gene Transfer (HGT) Protocols

The application was approved at Biosafety Level 2 containment (Standard and Universal Precautions) and sharps precautions for inoculating patients with the study drug, with the following additional requirements:

The protocol also has the following additional requirements:

- IRB Approval Letter. Please send the Yale IBC a copy of the current IRB Approval Letter for your IBC protocol file.
- Documentation of a start-up discussion of this protocol with representatives from YNHH that may include: nursing staff involved in the project, YNHH Occupational Health and Safety, YNHH Epidemiology and Infection Control, YNHH and/or Smilow Pharmacy, the research study team, and a patient advocate representative. Please contact Dr. Richard Martinello, Medical Director, Hospital Epidemiology and Infection Control at 203-688-4634 for the specific groups required to attend the start-up meeting for your protocol.

  You cannot begin the protocol until this start-up meeting is documented with YNHH Hospital Epidemiology and Infection Control.

- Any serious event that is both unexpected and associated with the use of the gene transfer product (i.e. there is reasonable possibility that the event may have been caused using the product) must be reported to the IRB of Record within 48 hours.
- A copy of the FDA IND authorization email accepting the initiation of the trial at Yale must be on file with the Yale IBC prior to the enrollment of patients in the trial. A copy of the latest version of your FDA-authorized protocol. If relevant, please describe any substantive differences between your current protocol and the protocol registered with the Yale IBC and approved by the FDA.
- A copy of the annual report to the FDA and the IRB must also be submitted to the Yale IBC for the protocol file.

The Yale IBC approval of your protocol will expire on the expiration date of the IRB protocol as reflected in the IRB approval letter. The IBC re-approval is contingent on the re-approval by the IRB of record. The protocol must be re-approved by the IRB of record at least annually.

Should you wish to add personnel to your project, change the scope or location of your work, you must notify the Biosafety Office. It is the responsibility of the Principal Investigator to train new personnel before they begin work.

Annual Reporting Requirements for Principal Investigators with Active Human Gene Transfer (HGT) Protocols

By the one-year anniversary of the approval date of a HGT Protocol, Principal Investigators must provide the Yale EHS Biosafety Office with an annual report that includes the following information.

1. Verification that the protocol is still active.
2. The date of the last Yale Human Investigation Committee authorization (annual HIC authorization is required for the continuation of an HGT Protocol).
3. For protocols that have been activated, a report that includes the number of subjects enrolled since the protocol has been initiated.
    a. This report must also include a summary of all adverse and serious adverse events reported for each enrolled patient in the last year identified by your team, another institution or the Sponsor.
    b. You may also provide the EHS Biosafety Office with an annual report from the Sponsor that includes this information.
4. Verification that each batch of the study drug shipped to Yale for use has been tested for adventitious agents and if applicable replication competent vectors.

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 (BSL1, BSL2, 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 coverslips, 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.

- 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.
- 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. Needle Box 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, diaminobenzidene (DAB), phorbol or phenol-chloroform mixtures are already chemically decontaminted. 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, diaminobenzidene (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, Handouts, and Additional Information

BSL1

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 BSL1 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.

BSL2 **Laboratory Practices**

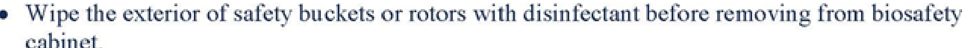
1. Keep laboratory door closed.
2. Post a universal biohazard label on equipment where infectious agents are used/stored.
3. Allow only persons informed of the research to enter BSL2 areas.
4. Keep animals not used in BSL2 experiment out of the laboratory.
5. Do not smoke, eat, drink, or store food in BSL2 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.

Centrifuge Safety

Yale *Environmental Health & Safety*

CENTRIFUGE SAFETY

- Each operator must be trained on the proper operating procedures.
- Keep a log detailing operation records for centrifuges and rotors.
- Do not exceed safe rotor speed.
- Place a biohazard label on the centrifuge if used for human material or infectious agents.
- Always use sealed safety buckets or sealed rotors with O-rings.
- 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 ¾ full.
- Always load and unload sealed safety buckets or rotors in the biosafety cabinet – never open them outside of the biosafety cabinet.
- 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.
- Move the sealed safety buckets or rotors in the biosafety cabinet before opening them.
- Decontaminate the safety bucket or rotor before removing from the biosafety cabinet.
- Decontaminate safety buckets or rotors and centrifuge interior after each use.
- Wash hands after removing gloves.



CENTRIFUGE SPILL

If a leak outside the safety bucket or rotor is noticed 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 EHS have given clearance (at least 30 minutes) • Follow centrifuge spill instructions in the Biosafety Manual, BSL3 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 EHS

Rev. 9/19

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).

Toxins

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

BSL2 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 BSL2 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 BSL2 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					
If using items below requirements indicated with an "X" must followed	**Biological General Registration 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)**
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 – BSL2	X		X	X							X				X	X	X			
Infectious Agents – BSL2+ and BL3	X		X	X								X			X	X	X			
Animal Use	X				X	X							X		X					
Animal use with BSL2 agent	X		X		X	X					X		X		X	X				
Animal use with BSL2+ 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						

NIH Dual Use Research Brochure

Where Can I Find out More Information?

Information about dual use research in the life sciences can be obtained from several sources. NIH OBA maintains a website on dual use research, including the findings and recommendations of the NSABB, as well as summaries and videocasts of NSABB meetings and other dual use-related videocasts at: www.biosecurityboard.gov

On this site, users can download an informative video describing the dual use issue from the perspective of leaders in science, research administration, and publishing as well as the public. In addition, your professional society, Institutional Biosafety Committee, or biological safety officer may also be a useful resource for questions pertaining to dual use life sciences research.

NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892-7985
Phone: (301) 496-9838
Fax: (301) 496-9839
Email: oba@od.nih.gov

Office of Biotechnology Activities

NATIONAL INSTITUTES OF HEALTH

DOES YOUR RESEARCH HAVE DUAL USE POTENTIAL?

AN OVERVIEW FOR INVESTIGATORS

What Is *Dual Use Research*?

Life sciences research is vital to improving public health, agriculture, and the environment, and to strengthening our national security and economy. Yet the very research designed to find ways to better the health, welfare, and safety of humankind can also yield information or technologies that could potentially be misused for harmful purposes. For instance, information from certain life sciences research could be misapplied to weaponize dangerous pathogens, to bypass or diminish the effectiveness of medical countermeasures, or to threaten in other ways the health and safety of humans, animals, plants and the environment. Research yielding new technologies or information with the potential for both benevolent and malevolent applications is referred to as "dual use research."

What Is *Dual Use Research of Concern*?

Some degree of dual use potential may be inherent in a significant portion of life sciences research. However, the small subset of life sciences research with the highest potential for yielding knowledge, products, or technology that could be misapplied to threaten public health or national security is referred to as "dual use research of concern."

Dual Use Research of Concern **is research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or materiel.**

What Are the Responsibilities of the Scientific Community?

Scientists have a professional responsibility to:

» Understand dual use research issues and concerns,

» Be aware of the implications of their work and the various ways in which information and products from their work could be misused, and

» Take steps to minimize misuse of their work.

What Is the National Science Advisory Board for Biosecurity (NSABB)?

The US Government established the NSABB to advise all Federal departments and agencies with an interest in life sciences research. The Board recommends specific strategies for the efficient and effective oversight of dual use life sciences research, as well as ways to raise awareness about the issue within the scientific community. The Board considers both national security concerns and the needs of the research community when providing guidance and recommendations to the Federal government.

The NSABB members include experts in science, medicine, law, security and public perspectives. The NSABB also includes nonvoting *ex officio* members from Federal agencies and departments with an interest in life sciences research.

The National Institutes of Health (NIH) Office of Biotechnology Activities (OBA) staffs the NSABB.