Yale University

Environmental Health and Safety

Biological Safety Manual

Environmental Health and Safety
135 College Street, Suite 100
New Haven, CT 06510
ehs.yale.edu

Procedure Following Exposure Incident

REPORT EXPOSURE IMMEDIATELY; YOU MAY NEED IMMEDIATE THERAPY.

Immediate Response:

- 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 (PI), manager or supervisor (if available) to initiate accident or exposure incident report
- Notify Yale EHS
- Seek medical assistance immediately (within 1-2 hours) from Yale Health, Acute Care
- Medical Area employees may also go to the YNHH Emergency Department or YNHH Campus and Occupational Health Services, located at 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 Campus and Occupational Health after receiving medical assistance from Yale Acute Care.

Emergency Phone Numbers

Police and Fire 911
Yale Health Acute Care 203-432-0123
EHS Emergency.....203-785-5555
YNHH Emergency Department.....203-688-2222
YNHH Campus and Occupational Health Services....203-688-2462
Yale Campus and Occupational Health 203-432-7978

1 Forward

This manual has been prepared as an update to the 1976 *Minimum Safety Guidelines for Biological Research at Yale*, and all previous versions of the *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.

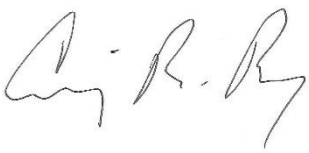
The manual focuses on Biosafety Levels (BSL) 1 and 2, as over 99% of Yale laboratories fall within these designations. A separate manual is available for researchers working in BSL-3 research laboratories. No work with BSL-4 agents may be conducted at Yale University.

The Yale Environmental Health and Safety (EHS) 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 (PI) or Supervisor to ensure that their laboratory is in compliance. That responsibility includes identification of the risks 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 (CDC)/National Institutes of Health (NIH) *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 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 EHS website. New editions or updates will be posted on our website as 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 questions or concerns about any aspect of the Biosafety Program at the University. The EHS training credos, “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 Office at 203-785-3550 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 R. Roy, Ph.D.
Chair, Yale Biological Safety Committee
Waldemar Von Zedtwitz Professor of Microbial Pathogenesis and of Immunobiology
Director, Biological and Biomedical Sciences

2 Introduction

2.1 Emergency Phone Numbers and Environmental Health and Safety (EHS) Contacts

Emergency Telephone Numbers

Police & Fire 911
EHS Emergency Line 203-785-3555
Police Dispatch 203-432-4400
Yale Health Acute Care 203-432-0123

Yale EHS Biosafety Staff Telephone Numbers

Yale EHS 203-785-3550
Benjamin Fontes, Biosafety Officer 203-737-5009
Maren Schniederberend, Biosafety Supervisor 203-737-7957
Anthony D'Abramo, Associate Biosafety Officer 203-737-2114
Julia Kontos, Associate Biosafety Officer 475-331-0650
Melissa James, BSL-3 Facility Manager 203-737-4228
Emily Bludnicki, Assistant Biosafety Officer 203-737-2127
Stephanie Monroe, Program Coordinator 475-414-7115

Yale EHS Website

2.2 Responsibilities

2.2.1 Department Chair

The Department Chair bears overall responsibility for conformity with biosafety rules and requirements and for the implementation and maintenance of safe practices and procedures in their department. They are responsible for ensuring that each Principal Investigator (PI) complies with Yale biosafety policies and applicable federal, state and local biosafety requirements pertinent to the research conducted under in their department. The Department Chair is also responsible for addressing repeat or persistent lab safety non-conformity identified among their staff.

2.2.2 Principal Investigator

The PI is responsible for ensuring conformity with all related policies, regulations, standards and guidelines that pertain to their research. This includes obtaining authorization for any regulated or hazardous research prior to initiation.

A large part of this responsibility is overseeing the conduct of research within their laboratories to ensure the safety of their researchers, others on the floor, within the building or in the community.

A PI must complete all required research registrations and provide a written risk assessment of their proposed work. The written risk assessment must include the following:

- Identification of all the biohazards and regulated biological materials utilized for their research.

    - This includes but is not limited to human, animal and/or plant pathogens, transmissible spongiform encephalopathies or prions, toxins, recombinant or synthetic nucleic acid (r/sNA) molecules, and field research.
    - The PI and all researchers in their laboratories must have a strong awareness of the biohazards, the diseases or toxicities associated with them and know the signs and symptoms of infection or poisoning for prompt reporting of illness to their PI and Yale Campus and Occupational Health (formerly Employee Health).
- Identifying all procedures to be used with biohazards and regulated biological materials in their laboratories, animal facilities, within specialized core laboratories, and in the field.
    - All the proposed work and all the locations where the work is conducted.
- Ensuring that prior to starting research, all personnel involved in their laboratories:
    - Have been listed on all applicable EHS, IACUC, and other registrations.
    - Have successfully completed all required safety and related training and retraining where applicable.
    - Have been evaluated for proficiency with the proposed research techniques and have been assessed and found competent with the commensurate biosafety work practices.
    - Have been provided with specific risk information and understand and are comfortable accepting the associated risks for their work in the lab. Risk information can be obtained from:
        - Pathogen Safety Data Sheets (PSDS) from Health Canada
        - Safety Data Sheets (SDS)
        - Agent summary statements from the CDC/NIH BMBL
        - Medical Surveillance summaries on Yale Biological Safety Committee approval letters
        - Other to be determined
    - Have been informed of personnel health risk factors associated with the research materials and have been provided with contact information and an opportunity for a private medical consult with Yale Campus and Occupational Health.
    - Where applicable, have been medically cleared by Yale Campus and Occupational Health to participate in the research.

The PI is also responsible for developing a set of safe working procedures for their proposed research with biohazards and regulated biological materials, or biosafety standard operating procedures (SOPs). The SOPs must include:

- The biosafety work practices required for the research;
- The personal protective equipment (PPE) required; and
- The engineering controls used to contain biohazards.

The PI must also ensure that the proposed facility research locations are commensurate with the proposed risk for their research.

- This includes ensuring that all elements of the biocontainment level required for the work, e.g., Biosafety Level 1 (BSL-1), Biosafety Level 2 (BSL-2), Biosafety Level 2-Enhanced (BSL-2+), and Biosafety Level 3 (BSL-3) where applicable, are met. If working at BSL-3, refer to the BSL-3 Manual for additional information.
- The PI is also responsible for maintaining their research equipment and ensuring that any facility concerns are promptly reported to Yale Facilities for evaluation and repair.

The PI must also ensure that their risk assessments are updated periodically, and whenever 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).

- Approval from the Yale Institutional Biosafety Committee (IBC) is required prior to initiation of any studies or research involving human, animal, or plant pathogens. The procedures for handling unclassified agents must also be reviewed by the Yale Biological Safety Committee and 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 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 PI must complete the "Registration of Research Involving r/sNA 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 r/sNA Molecules* (NIH Guidelines).
- Below is a table outlining the registration and training requirements for which the PI 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

	Required Registration								Required Training					Required Inspection					
If using items below requirements indicated with an "X" must followed	**Biological General (annual update)**	**rDNA**	**Biological Safety Committee**	**Connecticut Department of Public Health**	**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 (as requested by the IBC)**	**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			X					
Recombinant and Synthetic Nucleic Acid Molecules – non-exempt	X	X	X								X			X	X				
Infectious Agents – BSL-2	X		X	X							X			X	X	X			
Infectious Agents – BSL-2+ and BSL-3	X		X	X							X	X		X	X	X			
Animal Use	X				X	X					X		X	X					
Animal Use with BSL-2 agent	X		X		X	X					X		X	X	X	X*			
Animal Use with BSL-2+ or BSL-3 agent	X		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	X*	X		
USDA Regulated Agents (e.g., insects, plants)	X		X						X		X			X	X	X*		X	

*As applicable or as needed based on the agent

2.2.3 Research Personnel

Research personnel are the individuals handling potentially hazardous materials on a regular basis. Their understanding and adherence to safety policies is one of the most critical components of research safety compliance across the university. All research personnel are responsible for the following:

- Complete requirements for approval to work in the laboratory and ensuring 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 *BSL-3 Manual*, the *Exposure Control Plan*, the *Bloodborne Pathogens Training Manual*, or other applicable policies or manuals.
- 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 PI 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.
- Report any exposures, near-misses, or violations to the PI and Yale EHS.
- If inexperienced in handling human pathogens or tissue cultures, receive training and demonstrate proficiency in standard microbiological practices from the PI.
- 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:
    - Provide laboratory specific training for new staff members.
    - Discard biomedical waste containers appropriately before they become overfilled.
    - 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.
    - Regularly clean, disinfect, and organize individual work areas and common use areas.
    - Perform 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.
- Maintain supplies, including PPE.
- Maintain security of infectious agents (IA); i.e. store IA in a locked freezer in a locked laboratory, and maintain an inventory of all regulated, hazardous, or valuable biological materials.

2.2.4 EHS Office

The EHS Office provides:

- Information on laboratory operation to ensure compliance with CDC, NIH, OSHA, USDA, 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.
- Advice on safe methods for new procedures and provides advice in the event of large or high hazard biohazardous material spills.
- Assistance to laboratories for the preparation of city, state, federal or other regulatory inspections or visits.
- Assistance to laboratories with developing emergency response protocols for exposures, spills, or other incidents.
- Guidance on the design of new laboratory spaces for use of biohazards.

- An annual laboratory inspection program to help ensure continued compliance with safety regulations, standards, and Yale policies.

The Biological Safety Officer (BSO) is responsible for the implementation of policy guidelines recommended by the Yale Biological Safety Committee (Yale IBC). The BSO identifies potential problem areas and suggests to the Yale IBC safety objectives to be achieved. In addition, the BSO is also the institutional BSO for research with r/sNAs. Some of the specific biological safety services provided by EHS include:

- Evaluation and inspection of laboratory facilities for work with IAs and other hazardous biological agents;
- Investigation of laboratory accidents;
- Periodic updates of rDNA experiments to ensure compliance with the NIH Guidelines for Research Involving r/sNA 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 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.

2.2.5 Yale Biological Safety Committee

The Yale Biological Safety Committee shall serve as the IBC as defined in the National Institutes of Health (NIH) *Guidelines for Research Involving r/sNA Molecules (NIH Guidelines).* As such, the IBC shall review applications for research involving r/sNA to determine whether the facilities, procedures, and practices meet the standards required by the university and the NIH. The IBC 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 by posting on an accessible website as required by the NIH Guidelines.

The IBC's responsibilities include:

- Reviewing rDNA and IA 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 IBC 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 IBC shall also recommend guidelines relating to procedures and facilities used at the university, including such matters as safety training and health surveillance.

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

The IBC 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 IBC may find desirable.

3 Biosafety Requirements

The following information describes the requirements for Yale researchers as defined by the Yale Biological Safety Committee (IBC) and the Biosafety Office. It is the responsibility of each Principal Investigator (PI) to ensure the laboratory is in compliance with applicable regulations, standards, and Yale policies. All registrations regarding the use of biological materials are maintained through EHS Integrator.

3.1 Biological General Registration

All PIs who handle biological materials are required to complete and submit a Biological General Registration through EHS Integrator. Environmental Health and Safety (EHS) must maintain accurate information regarding the use of biological materials (e.g., microorganisms, cell lines, human materials, animals, and toxins) by all university personnel. EHS policy requires all PIs 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 laboratory or pathogen storage 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 (r/sNA) Molecules (NIH Guidelines)*, and the Connecticut Department of Public Health (CT DPH). EHS will assist in updating information during the annual biological and chemical safety lab inspection.

The Biological General Registration is accessible at EHS Integrator Registrations.

3.2 Human Pathogens

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

- Yale Institutional Biosafety Committee (IBC)
- Connecticut Department of Public Health (CT DPH)

To initiate these approvals, complete and submit an Infectious Agent (IA) Registration available at EHS Integrator. EHS staff will perform a preliminary review of the registration. Then a member of the Biosafety Office presents the registration to the IBC for review, and the IBC will vote on the registration. Biosafety Office staff will notify the PI, lab contacts, and assistants of the committee's decision and provide guidance on any outstanding contingencies.

If the lab space is not currently registered with the CT DPH, Biosafety Office staff will notify the CT DPH and arrange for inspectors from the CT 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 IAs or potentially hazardous biological materials into animals also require approval from the:

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

Many etiologic agents have been assigned a risk group (RG) level based on the degree of hazard they pose. 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.

RG2 and higher etiologic agents are listed in the NIH Guidelines in Appendix B. Call the Biosafety Office at 203-785-3550 for assistance with agents that are not listed.

All researchers working with etiologic agents (RG2 or higher) must receive training in both biosafety and the microbiological procedures that will be utilized for the experiment. Biosafety training courses are available on the EHS website. The PI is responsible for ensuring that all researchers are trained in the appropriate procedures and techniques used in the laboratory, complete all initial and refresher training requirements, and receive appropriate medical surveillance.

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

Contact your Safety Advisor (SA) **before**:

- Initiating work with a new IA.
- Modifying your research in a manner that enhances the risk.
- Changing the location of existing work.
- Adding researchers to your IA registration.
- Providing IAs, other regulated biological materials, or other regulated biohazards to another investigator on or off campus.
- Arranging for any visiting researchers to work in your laboratory.

Work with RG3 agents requires additional training and documentation. Contact the Biosafety Office at 203-785-3550 and refer to the *Biological Safety BSL-3 Laboratory Manual* for additional information.

3.3 Experiments with recombinant or synthetic nucleic acid molecules (r/sNA)

Yale IBC approval is required prior to the initiation of non-exempt r/sNA experiments. A brief description of non-exempt and exempt r/sNA 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 2024*.

Contact an EHS staff member if you have questions on how to:

- Register non-exempt r/sNA work.
- Modifying your r/sNA research in a manner that enhances the risk.
- Changing the location of existing r/sNA work.
- Adding researchers to your rDNA registration.
- Inquire about any aspects of recombinant work with r/sNA.

The rDNA registration is accessible via EHS Integrator. The NIH guidelines are available online.

3.4 Human Gene Transfer

Proposed clinical trials involving Human Gene Transfer (HGT) require registration and approval from both campus and federal agencies before initiation. The Yale University Institutional Biosafety Committee (IBC) requirements for HGT protocols are detailed in the Guidelines for Human Gene Transfer Clinical Trials which encompasses details surrounding such trials, submission instructions, and the HGT registration form. Visit the EHS website for more information about HGT protocols at Yale.

Learn more: National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

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

The OSHA created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard) to minimize or eliminate exposure to IAs that may be present in human blood, tissues or certain body fluids (bloodborne pathogens.) The Bloodborne Pathogens Standard applies to all employers with employees that are exposed to human blood or other potentially infected materials (OPIM). Employees with a reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or OPIM in the performance of their duties are considered occupationally exposed. OPIM includes:

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

Please note, these are the body fluids identified in the OSHA BBP standard but be aware that all body fluids with the exception of sweat may harbor a human pathogen.

An individual is also considered occupationally exposed if they use equipment that is used to process or store blood or OPIM if the equipment has not been decontaminated before their use.

All occupationally exposed employees are required by OSHA to attend a BBPs training session prior to beginning work and within 365 days of their prior training. These employees must also be offered the HBV vaccination within 10 days of hire. 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 BBPs such as:

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

Please note this list is not all encompassing. Consult the *Bloodborne Pathogen Training Manual* and the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* (BMBL) for additional information including more comprehensive lists. Contact the Biosafety Office at 203-785-3550 for assistance with exposure determination and training information.

3.6 Animals

All research experiments involving vertebrate animals must be conducted in accordance with the associated Yale IACUC approved protocol. Animal research that involves a hazard (biological, radiological, or chemical) must be reflected in the approved IACUC protocol. Medical clearance must also be obtained prior to initiating work in animal areas. The level of clearance required depends on the types of animals that will be encountered. Contact the IACUC at 203-785-5992 and Campus and Occupational Health at 203-432-7978 for additional information.

Once work with hazards is approved by IACUC, the Office of Animals Research Support (OARS) must be contacted prior to initiation of animal research to ensure that a safety protocol will be established. Researchers must meet with OARS and YARC personnel and the relevant safety personnel to outline standard operating procedures (SOP). An orientation to the assigned animal housing area will be provided. Call OARS at 203-785-5992 for additional information.

The Biosafety Committee must approve work with human pathogens and/or r/sNA in animals (including transgenic animals) prior to initiation. Contact the Biosafety Office at 203-785-3550 to initiate the approval process for the IBC.

For additional information please contact the IACUC at 203-785-5992 or YARC at 203-785-2526.

3.7 Biological Safety Cabinets (BSCs) and Other Clean Air Devices (CADs)

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

The efficacy of biological safety cabinets (BSC) and other CADs depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory. Please note that it is prohibited for two individuals to work simultaneously in a single BSC.

All BSCs and CADs at Yale must be placed on the Yale University certification/service contract and be certified at least annually. Any BSCs or CADs not under the certification/service contract will be placed in storage status. EHS may contact you to schedule the required annual certification if you have not been contacted by Yale University's CAD vendor.

Notify the Biosafety Office in advance when you plan to have BSCs or CADs moved, placed in storage, transferred to a new owner, discarded, removed from Yale or obtained from another institution or manufacturer. Contact the CAD@yale.edu if service or repairs (e.g., replacing fluorescent lamps, switches, etc.) are needed for your unit. BSCs must be professionally decontaminated by a qualified technician of a Yale University approved vendor before a unit is relocated, stored, serviced (interior) or discarded.

The purchase of BSCs and other CADs is coordinated through Yale University Purchasing Department and the Biosafety Office. The Biosafety Office reviews all BSC and CAD purchase requests. Contact the Biosafety Office for more information. Yale actively discourages the purchase and use of laminar flow clean benches (LFCBs) since air is blown across the work surface into the face and torso of the operator. The IBC and the Biosafety Office recognize that LFCBs do not provide personnel or environmental protection from infectious or potentially IA, allergens, chemicals or radioactive materials. If you are using a LFCB, 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 website or contact the Biosafety Office at 203-785-3550.

3.8 Toxins

Toxins come under the purview of the EHS Biosafety Office when dealing with toxins of biological origin. As they are not infectious, they are essentially a "chemical" and instead of dealing with infectivity, we are evaluating its toxicity, usually in the form of the toxin's lethal dose to 50% of the species exposed (LD50) values.

LD50s are used to classify toxins based on risk by various groups, including the NIH Guidelines, the BMBL, the United States Policy on Dual Use Research of Concern (DURC), the Select Agent Regulations, Shipping and Transport Rules, and chemical safety guidance documents.

PIs and researchers wishing to order, obtain, or conduct research with toxins must be aware of the following regulatory requirements.

3.8.1 Purchasing Toxins at Yale

Toxins are a restricted purchase item and must be approved by Yale EHS prior to ordering. EHS will check to see what you are using the toxin for, how much you will order, and verify that you have all the relevant toxin safety information and if applicable, registrations required for toxin research. EHS has developed a toxin safety poster that must be placed in lab areas where toxins are used or stored. An EHS representative will meet with you to make sure those handling toxins understand the required safety protocols.

If you are working with toxins in animals, EHS will ensure the toxin is on your current IACUC protocol.

If you are conducting r/sNA research involving toxins, EHS will ensure that you already have approval for this research or may reach out for the completion of additional biosafety registrations prior to initiation.

EHS will ensure relevant lab safety and biosafety training for those who will be working with toxins are complete.

3.8.2 Possession of Select Agent Toxins

EHS will also determine if you are going to be ordering, storing, and/or working with any of the nine Select Agent Toxins, which are more regulated than other toxins. The table below shows the nine Select Agent Toxins and the maximum quantity allowed to be in the possession of a single PI (aggregate amount of all of the toxin in all of the PI's lab spaces). Any exceedance of these quantities will require a very involved U.S. Government Select Agent registration, which will include significant safety documentation along with a federal inspection prior to being allowed to possess these quantities for biosecurity purposes. Please let EHS know if you are planning to be in possession of any of the amounts associated with the required government registration. The Yale Watch List quantity is 50% of the volumes allowed by the federal government, and at this level of possession, EHS will be periodically assessing your quantities with you to ensure that you do not exceed these amounts accidentally, as the penalties for doing so are extreme. The table below is an example of an inventory form that Yale PIs who are in possession of any of the nine Select Agent toxins must use and retain in their laboratories.

Select Agent Toxin	Maximum allowed Quantity (1)	Yale University Watch List Quantity (2)	Total mg Aggregate Quantity in all of PI's labs (3)
Abrin	1000 mg	500 mg	
Botulinum neurotoxins	1 mg	0.5 mg	
Short, paralytic alpha Conotoxins	200 mg	100 mg	

Diacetoxyscirpenol (DAS)	10,000 mg	5,000 mg	
Ricin	1000 mg	500 mg	
Saxitoxin	500 mg	250 mg	
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg	50 mg	
T-2 toxin	10,000 mg	5,000 mg	
Tetrodotoxin	500 mg	250 mg	

(1) Possession of quantities greater than the maximum quantity allowed under the Select Agent exemption requires registration and authorization from the federal government (CDC). **PIs are not allowed to possess greater than the quantities listed above at any time. Possession of quantities above the limit is a violation of the Select Agent regulations and requires immediate notification. PIs and the University are subject to financial penalty if these quantities are exceeded without federal authorization.**

(2) The Yale Watch List Quantity is simply 50% of the federally allowed exempt quantity. PIs who reach the watch list quantity will be sent a periodic inventory log to help ensure that the quantity of the toxin they possess never exceeds the federal exempt quantity.

(3) This is the total aggregate quantity of each Select Agent toxin under the possession of a single PI at Yale University. It is the sum of the quantity of the toxin in all of their laboratories. For example, if a PI has 12 bottles of one of the toxins listed above in their seven research lab rooms, then the quantity of toxin in all 12 bottles would be added to identify the total aggregate quantity. **Please note: Toxin suppliers are allowed to sell labs up to the federal exempt quantity limit of each toxin. If you purchase the maximum quantity, please ensure that you have zero mg of that toxin in your laboratory prior to submitting the order.**

3.8.3 Toxin Due Diligence Requirements in U.S. Government Select Agent Regulations

All Yale PIs in possession of **any quantity** of the nine Select Agent toxins listed above must retain a record of **all** transfers of **any quantity** of these toxins outside of their laboratory. **This includes transfers to other Yale laboratories**. This requirement is to document the recipient's purpose of use and ensure the recipient is authorized to possess the toxin. EHS has created an inventory form that Yale PIs can use to satisfy this requirement.

3.8.4 Reporting Suspected Violations or Suspicious Activity Involving Toxins

If a Yale PI in possession of **any quantity** of the nine Select Agent Toxins listed above detects suspicious activity associated with a request for toxin or suspicious activity associated with a shipped toxin, they must immediately notify the Yale University Responsible Official (RO) or Alternate Responsible Official (ARO) and the Federal Select Agent program (FSAP). Contact information for key EHS personnel you can report to are provided below.

Yale EHS Executive Director	Kevin Charbonneau (ARO)	203-737-2139 (office)	203-410-8527 (cell)
Yale EHS Senior Associate Director	Ben Fontes (RO)	203-737-5009 (office)	203-410-6223 (cell)
Yale BSL-3 Manager	Melissa James (ARO)	203-737-4228 (office)	412-551-9068 (cell)
Yale EHS Emergency Line		203-785-3555	

To report fraud involving the Select Agent program, contact the office(s) listed below.

Email CDC	LRSAT@cdc.gov	
CDC Select Agent Office	404-718-2000	
Anonymous HHS OIG Hotline	1-800-447-8477 (voice)	1-800-223-8164 (fax)
Anonymous USDA OIG Hotline	202-690-1622 (voice)	202-690-2474 (fax)

Performing Recombinant or Synthetic Nucleic Acids (r/sNA) Research with Toxins

The categories of r/sNA related to toxins is provided below, directly cut and pasted from the April 2024 *NIH Guidelines for Research Involving r/sNA Molecules.*

Section III-B. Experiments That Require NIH Office of Science Policy and Institutional Biosafety Committee (IBC) Approval Before Initiation

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH OSP. The containment conditions for such experiments will be determined by NIH OSP in consultation with ad hoc experts. Such experiments require IBC approval before initiation (see Section IV-B-2-b-(1), IBC).

Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of r/sNA molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the OSP, National Institutes of Health (NIH), preferably by submitting a request for this information to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on the NIH OSP website.

APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

Appendix F-I. General Information

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD50 of < 100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under Section III-B-1 (Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms Per Kilogram Body Weight) and require IBC and NIH OSP approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD50 ≥ 100 micrograms per kilograms of body weight. Experiments involving genes coding for toxin molecules with an LD50 of < 100 micrograms per kilograms and > 100 nanograms per kilograms body weight require IBC approval and registration with NIH OSP prior to initiating the experiments. A list of toxin molecules classified as to LD50 is available from NIH OSP. Testing procedures for determining toxicity of toxin molecules not on the list are available from the OSP, NIH, preferably by submitting a request for this information to: NIHGuidelines@od.nih.gov; additional contact information

is also available here and on the NIH OSP website. The results of such tests shall be forwarded to NIH OSP, which will consult with ad hoc experts, prior to inclusion of the molecules on the list (see Section IV-C-1-b-(2)-(c), Minor Actions).

Appendix F-II. Cloning of Toxin Molecule Genes in *Escherichia coli* K-12

Appendix F-II-A. Cloning of genes coding for molecules toxic for vertebrates that have an LD50 of >100 nanograms per kilograms and <1000 nanograms per kilograms body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under Biosafety Level (BSL) 2 + EK2 or BL3 + EK1 containment conditions.

Appendix F-II-B. Cloning of genes for the biosynthesis of molecules toxic for vertebrates that have an LD50 of >1 microgram per kilogram and <100 microgram per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, Bordetella pertussis toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

Appendix F-II-C. Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *Escherichia coli*, *Klebsiella*, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *Escherichia coli* and of *Yersinia enterocolitica*.

Appendix F-III. Cloning of Toxic Molecule Genes in Organisms Other Than *Escherichia coli* K-12

Requests involving the cloning of genes coding for toxin molecules for vertebrates at an LD50 of <100 nanograms per kilogram body weight in host-vector systems other than *Escherichia coli* K-12 will be evaluated by NIH OSP in consultation with ad hoc toxin experts (see Sections III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms Per Kilogram Body Weight, and IV-C-1-b-(2)-(c), Minor Actions).

Appendix F-IV. Specific Approvals

An updated list of experiments involving the deliberate formation of r/sNA molecules containing genes coding for toxins lethal for vertebrates at an LD50 of <100 nanograms per kilogram body weight is available from the OSP, NIH, preferably by submitting a request for this information to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on the NIH OSP website.

3.8.5 Toxins and the National DURC Policy for NIH Funded Institutions

Currently, there is only a single toxin, Botulinum toxin, listed as a restricted agent, that requires review by the Yale University Institutional Review Entity (IRE), prior to any use, regardless of quantity, in a clinical, animal, or research protocol. Please contact Yale Biosafety Office if you are planning to work with Botulinum toxin in human subjects, animals, or in your lab research projects. Even FDA-approved botulinum products still require this review by the Yale IRE.

3.8.6 Discovery of a Toxin

To report the finding of an unknown or unexpected toxin in your laboratory or freezer, immediately notify Yale EHS Emergency at 203-785-3555. Please provide the name of the toxin and the quantity listed on the label if known. Please keep the toxin in a secure protected location until EHS arrives. EHS will work with your lab on the appropriate next steps based on the toxin and the amount.

3.9 Training

Successful completion of a range of biosafety training programs may be required prior to the initiation of your research at Yale University. Please access the required safety trainings assigned to you in Workday Learning to ensure compliance. For reference, a full list of all offered biosafety training programs can be found on the EHS website. If you have any questions, please contact the EHS Training Coordinator at safetytraining@yale.edu.

Additional Notes:

- There are a variety of Bloodborne Pathogens training versions dependent upon your role and scope of work at Yale. Your Workday Learning profile will assign you the correct version. OSHA requires Bloodborne Pathogens training to be completed annually.
- For researchers working with Infectious Agents in a CT DPH registered laboratory, the DPH Inspection Program (encompasses Biosafety parts I and II) training is required every 5 years.
- For specifics regarding BSL-3 training requirements (hands-on training, observations, etc.), please contact safetytraining@yale.edu for more information. Additionally, refer to the Biosafety Level 3 manual.

4 Medical Surveillance Program

A medical surveillance program for Yale University personnel engaged in biological research is administered by Campus and Occupational 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 as well as their personal risk factors. For a particular employee, the medical surveillance program might call for any of a number of precautionary measures, including (but not limited to) immunizations, a periodic examination, and/or collection of a serum specimen. See the EHS Medical Surveillance Requirements Fact Sheet for more information.

The purpose of the medical surveillance program is to:

- Recommend appropriate medical precautions to be followed, and
- Perform 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 and procedures performed;
- The biological agents to which the individual is directly or potentially exposed; and
- Certain additional factors relating to the current or previous health status of the individual.

The Principal Investigator (PI) is to provide Campus and Occupational 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 Yale Health Center Campus and Occupational Health Department at 203-432-7978.

4.1 Tuberculosis (TB) Screening

Employees identified as having occupational exposure to Tuberculosis (TB) are enrolled in the University's Tuberculosis Exposure Control Plan. The Occupational Safety and Health Administration (OSHA) 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 *Mycobacterium tuberculosis* (TB).

New employees at risk must be tested for TB exposure by a tuberculin skin test (PPD) or QuantiFERON Gold at time of hire (within 2 weeks of start date) to establish a baseline. Additionally, they must complete a TB risk assessment and symptom screening questionnaire.

Initial and periodic (at least annual) TB surveillance is also applicable to individuals who may have contact with or enter facilities housing non-human primates (NHP), and those who may handle unfixed NHP tissues or samples, and after any exposure to NHPs or NHP material(s).

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 Campus and Occupational Health at 203-432-7978 to arrange for testing and for additional information regarding the University's TB Exposure Control Plan embedded within the University's Infection Control Manual. Contact EHS at 203-785-3550 for information on TB training.

4.2 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**
Arboviruses: • Eastern and Western Equine Encephalitis virus. • Japanese Encephalitis virus. • Rift Valley Fever virus. • Venezuelan Equine Encephalitis virus. • Yellow Fever virus.	Prior to working with arboviruses, employees are **required** to receive a medical evaluation and counseling from Campus and Occupational Health regarding possible immunization.
Borealpox (Alaskapox) virus	Researchers that will be working with Borealpox virus or working in a room where Borealpox virus is used, **must** meet with the Campus and Occupational Health Physician by calling 203-432-7978 for a medical evaluation and to discuss receiving vaccination before initiating experiments with Borealpox virus. The current preferred vaccination is Jynneos, which must be requested by the CT DPH through CDC based on the specific research project.
Chikungunya virus	Chikungunya virus immunization is **recommended** for those directly handling the virus. Immunization is offered to anyone who may enter the room while the work is in progress (in the Biosafety cabinet (BSC) or in the incubator, or in animals if applicable), or who handles/treats bagged waste containing these materials. Contact Yale's Campus and Occupational Health Office at 203-432-7978 to receive the vaccine. Pregnant women or people who are contemplating pregnancy are not recommended to handle this agent. Please schedule a medical consult with the Campus and Occupational Health Physician by calling 203-432-7978.
Hepatitis B virus (HBV)	**Recommended** for personnel working with human blood, body fluids or tissues.

Highly Pathogenic Avian Influenza (HPAI) virus	Annual seasonal influenza vaccination is **required** for personnel entering a room where HPAI is stored or handled. Contact Yale's Campus and Occupational Health Office at 203-432-7978 to receive the influenza vaccine.
Influenza A and B virus	The annual influenza vaccine is **recommended** for all personnel handling Influenza virus. Contact Yale's Campus and Occupational Health Office at 203-432-7978 to receive the influenza vaccine.
Measles virus	All people working in the lab **must** contact Yale's Campus and Occupational Health Department proof of an up-to-date Measles vaccine or have a measles titer done to confirm immunity to Measles, is **required**. An up-to-date Measles vaccine consists of two vaccine doses since 1980 and after at least 12 months of age, or a positive titer. Contact Yale's Campus and Occupational Health Office at 203-432-7978 to complete this requirement.
Monkeypox/Mpox virus (Clade II, non BSAT and Clade I, BSAT)	Monkeypox/Mpox immunization is **required** for those directly handling the virus. Monkeypox immunization is offered to anyone who may enter the room while the work is in progress. Contact Yale's Campus and Occupational Health Office at 203-432-7978 to receive the vaccine.
Mumps virus	All employees handling mumps virus are **required** to have evidence of 2 mumps vaccinations. If vaccine documentation is unavailable, then a *positive* Mumps titer (confirming immunity) will suffice.
Neisseria meningitidis	All personnel working with *Neisseria meningitidis* **are required to be evaluated and cleared** by Yale Campus and Occupational Health before beginning any work with this agent. Establishing a medical history with Campus and Occupational Health is imperative to ensure there are no underlying conditions, such as asplenia, complement deficiency, or immunosuppression, that could increase the risk of invasive meningococcal disease. Campus and Occupational Health **requires** completion of a vaccination series with a quadrivalent meningococcal vaccine for meningitis ACWY prior to working with those strains. A booster is **required** every 5 years after completing the primary series. Campus and Occupational Health also requires completion of a vaccination series for meningitis B prior to working with that strain. A booster is **required** every 2 years. The booster must be the same product (either Trumemba or Bexero) that was used for the primary series. Campus and Occupational Health will provide this evaluation and any necessary vaccinations at no cost to the employee. In the event of common symptoms of laboratory-acquired infection such as fever, headache, neck stiffness, or petechial rash, it is crucial to promptly report to Yale Campus and Occupational Health for evaluation. Early recognition is vital for survival. Furthermore, any laboratory accident, including needlesticks, splashes, or aerosol exposures, should be immediately reported for medical evaluation and potential

	antimicrobial chemoprophylaxis. Call Campus and Occupational Health at 203-432-7978 to schedule appointments or for any questions.
Other vaccines such as for *Salmonella typhi*	To be determined on a case-by-case basis by the Campus and Occupational Health Provider.
Rabies virus	Rabies immunization **required** for those directly handling the virus, animals infected with the virus, or untreated waste. Rabies immunization is **recommended** to anyone who enters the room while rabies virus research is in progress (e.g., in the BSC, in the incubator). Contact Yale's Campus and Occupational Health Office at 203-432-7978 to receive the vaccine.
SARS-CoV-2 (COVID-19)	Laboratory researchers who work with SARS-CoV-2 are **recommended** to receive the current season's COVID-19 vaccine, regardless of prior vaccination or infection history. (As of 07/17/2025, this is 2024–2025 formulation that targets the most prevalent circulating Omicron sub lineages, e.g., JN.1, KP.2, KP.3, XEC.) Additional doses may be recommended for adults aged =65 years and for those who are moderately to severely immunocompromised. This could change based on the Yale Biological Safety Committee's risk assessment of individual SARS-CoV-2 and related coronavirus research experiments in the future. Follow the established University isolation protocols if personnel have symptoms of COVID or test positive and report any positive COVID test to Campus and Occupational Health.
Streptococcus pneumoniae	Personnel that will work with *Streptococcus pneumoniae* are **recommended** to receive Pneumovax. To receive the vaccine, contact Yale Campus and Occupational Health at 203-432-7978.
Vaccinia virus	Researchers that will be working with Vaccinia virus or working in a room where Vaccinia virus is used, are **required** to meet with the Campus and Occupational Health Provider for a medical evaluation and to discuss receiving vaccination before initiating experiments with Vaccinia virus. The current preferred vaccination is Jynneos, which must be requested by the CT DPH through CDC based on the specific research project. Pregnant women or people who are contemplating pregnancy are not recommended to handle this agent. Please schedule a medical consult with the Campus and Occupational Health Provider by calling 203-432-7978.
Yellow fever virus strain 17D	The Yellow Fever vaccine is **recommended** for all researchers handling Yellow Fever virus strain 17D. Contact Yale Campus and Occupational Health Office at 203-432-7978 to receive the vaccine.

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

4.3 Medical Restrictions

4.3.1 Pregnancy

It is recognized that exposure to certain infectious agents (IA) 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 IAs you should consult your PI or supervisor. The Department of Campus and Occupational Health is also available for questions regarding the potential harm from the biological agents present within your laboratory.

Pregnant women or people who are contemplating pregnancy are encouraged to inform their supervisors or PIs and Campus and Occupational Health. *Employees are urged to discuss exposure issues with their supervisors or PIs regarding associated risks of research being conducted and pregnancy*. Campus and Occupational Health will give advice about precautions that might be necessary.

Campus and Occupational Health is a resource for pregnant women and people who are contemplating pregnancy to ask about any questions or concerns they may have regarding risks in their work environment. Campus and Occupational Health may also act as a liaison to make recommendations to the respective supervisors or PIs.

If there is a determination made that the pregnant person is recommended to be restricted from parts of their work duties or would like to request accommodations that have been recommended by their own provider, then Campus and Occupational Health will reach out to the Office of Institutional Equity and Accessibility and notify them of the need to engage in the interactive process with the patient. Since the pregnancy would be classified as the patient's own medical condition (not a work-related condition), then documentation from their own provider outlining the request will likely be required.

4.3.2 Reproductive Biological Hazards

The Campus and Occupational Health Provider will offer confidential counseling to any woman or man of childbearing age working with reproductive pathogens or other potentially infectious materials (OPIM). Reproductive biological hazards include, but are not limited to the following:

- *Babesia microti*
- *Borrelia hermsii*
- *Brucella spp.*
- Chikungunya virus
- *Coxiella burnetti*
- Cytomegalovirus
- Dengue virus
- *E. coli* (enterotoxigenic)
- Echovirus 24
- Enteroviruses
- *Haemophilus influenzae*
- HBV
- Hepatitis E virus
- Herpes Simplex virus
- HIV
- Human Papilloma virus
- Japanese Encephalitis virus
- *Klebsiella pneumoniae*
- *Leptospira spp.*
- *Listeria monocytogenes*
- Lymphocytic Choriomeningitis virus
- *Neisseria gonorrhoeae*
- Oropouche virus
- Parvovirus B19
- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Salmonella typhi*
- *Staphylococcus aureus*
- *Treponema pallidum*
- *Ureaplasma urealyticum*
- Vaccinia virus
- Venezuelan Equine Encephalitis virus
- Zika virus

Whenever necessary, Campus and Occupational 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 do not involve exposure to the known reproductive hazard (generally with actual pathogens, not necessarily for only OPIM such as blood or body fluids) will be discussed and can be reviewed per university human resources policies. Also, PIs actively working with reproductive hazards are to explain the risk assessment at time of hire.

4.3.3 Other Restrictions

Restrictions or recommendations will be made on an individual basis after discussion with the Campus and Occupational Health Provider 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 experiencing any of the above conditions, you must inform your physician and Yale Campus and Occupational Health about the situation.

4.4 Employee Serum Storage

Many infections do not result in an overt disease condition. Such infections are detected by the development of antibodies to the agent in question. Therefore, Campus and Occupational Health has established a program for personnel engaged in Biosafety Level 3 (BSL-3) research, which includes collection of serum prior to beginning the research. Additional serum samples will be collected if an illness occurs which may be related to the agent the person is working with.

5 Accidents

5.1 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.

5.1.1 Percutaneous Injury

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

5.1.2 Splash to Face

Flush affected area in eyewash for 15 minutes.

- See eyewash videos for instructions for use.

5.1.3 Aerosol Exposure (for labs working at BSL-2 or higher)

- Hold your breath and immediately leave the 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.
- Notify your PI/supervisor.
- PI/supervisor must clear lab for re-entry.
- For extensive BSL-2 contamination (i.e., centrifuge incident) or incidents involving RG2 or RG3 agents, Yale Environmental Health and Safety (EHS) must be notified and will assume responsibility in conjunction with the PI to clear the laboratory for re-entry.

5.2 Reporting Incident

The employee must promptly report the incident to their supervisor and EHS.

After you have been seen by a health professional, report a claim to CorVel at 1-833-400-7222. For additional information, see Workers' Compensation.

If the injured person is a visitor or student, report the incident to the Office of Risk Management via email and report the claim to the claims administration company.

5.3 Medical Assistance

Employees are urged to call Campus and Occupational Health at 203-432-7978 after they have received proper available first aid at site of exposure. For certain exposures such as non-human primate (NHP) bites or scratches, tick or insect bites, or exposure to infectious agents (IA) or human materials, the employee will be advised to come in and be evaluated by Campus and Occupational Health. In situations when Campus and Occupational 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 Campus and Occupational Health.

Campus and Occupational Health 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 IA(s).

Employees can obtain copies of their medical records by contacting Yale Health Information Management (HIM) at 55 Lock Street by phone 203-432-0062 or email. Yale University must retain medical records for your duration of employment plus 30 years.

5.4 Investigation of Laboratory Accidents

Yale EHS, in cooperation with the PI and their staff, will conduct the necessary investigation of a laboratory accident. The goal of the investigation is to prevent similar accidents as well as obtain information concerning the circumstances. In addition, Yale EHS, in consultation with Campus and Occupational 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 PI/supervisor is the responsibility of the employee who has had the accident. The PI/supervisor should then report the incident to Campus and Occupational Health at 203-432-7978. 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.), EHS must perform an investigation to determine if a safe sharps device is available, the root cause of the incident, and if the injury was a result of a policy deviation. If safe sharps devices are available, they must be evaluated by EHS in conjunction with the laboratory group. The incident must also be recorded on the University's Sharps Injury Log, maintained by EHS. The confidential log will include the type and brand of device involved in the incident, the laboratory area where the exposure incident occurred, and an explanation of how the incident occurred.

6 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 Yale Biological Safety Committee (IBC) and Biosafety Office the authority to administer the campus biosafety program. The IBC establishes policies for the safe use of biohazards and for compliance with all applicable regulations. As an extension of the IBC, 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 (PI) 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 IBC approval prior to initiation (See Section 3).

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.

Further information on the 6 Ps of Risk Assessment and Risk Management is discussed below.

Risk Assessment

1. **Pathogen** review and properties of the hazardous biological agent.
2. Review of all proposed **procedures** and experimental manipulations with the biohazard.
3. **Personnel** evaluation including appropriate training and skills.

Step 1:

Pathogen – Gather all information on the biohazard or agent:

- Download the Pathogen Safety Data Sheet (PSDS) from the Canadian Public Health website.
- Obtain the Agent Summary Statement from the CDC/NIH BMBL if available.
- Understand the signs and symptoms of infection.
- Know all clinical syndromes that can be caused by the agent.
- Identify the incubation period, the infectious dose, the starting Risk Group (RG).
- Know the specific strain/variant of the organism.
- Know the susceptibility of the organism to commonly used therapeutic drugs and identify any effective immunizations, including if the organism is multi-drug resistant or difficult to treat.
- 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 Campus and Occupational Health prior to starting work with biohazards for a private consult regarding their health status.
- 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 (SOP)).
- 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.). Please consider the following when developing SOPs:
    - Pay particular attention to possible injuries from contaminated sharps:
        - If not working with animals, eliminate sharps and use plastic.
        - When a sharp is required, select needles and sharps with engineered safety features (e.g., retractable, self-sheathing) whenever available and appropriate for the procedure.
    - Any step with a liquid can generate droplets that could make contact with facial mucous membranes, skin, or personal clothing and contaminate surrounding work areas.
    - All procedures involving biohazardous liquids may generate aerosols. These include but are not limited to vortexing, homogenizing, pipetting, and centrifugation.
- Risks from animal experiments include puncture injuries from inoculation, bites and scratches, lab animal allergens, and exposure to bedding contaminated from excretion of the biohazards.
- Identify the potential for spills or releases such as dropped flasks, broken flasks in shakers, leaks in centrifuges, etc.
- Document in writing all the steps and risks identified and detail the potential for exposure. Please note, this is your required written site-specific risk assessment!

PAUSE: Ask the EHS Biosafety Office or your assigned Safety Advisor (SA) to review your risk assessment at this point. Other resources that are available include the IACUC, IBC, and IRB. The EHS Biosafety Office will confirm your risk assessment and help identify any procedures with potential risks that may have been missed. Update your written risk assessment after review.

Step 3:

Personnel Evaluation – review who will be handling biohazards:
Please ensure the following questions are addressed prior to initiating work:

- 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 by the EHS Biosafety Office with an experienced researcher.
- Have applicable staff completed all required Biosafety and other 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 associated with the proposed research and procedures?
    - Are any staff at greater risk due to their health status?
        - Have they met with and been cleared by Campus and Occupational 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?
        - If yes, has a suitable treatment been identified for them?
- Are all researchers comfortable risks associated with the proposed work? Any researchers with concerns may contact Campus and Occupational Health for a private discussion.
- Have you documented the proficiency of the staff with the lab protocols and the associated biocontainment practices required to mitigate risks? You may utilize the EHS Researcher Competency Verification form for this step.

Risk Management

- Formulation of work **practices** to mitigate identified risks.
- Selection of **protective equipment,** including protective clothing and safety equipment to place barriers between staff and the biohazard.
- Utilization of a **place** or laboratory that is suitable for the proposed research.

Step 4:

Practices – review the work practices that will be performed throughout the experiment**:**

Biosafety work practices will help to reduce or minimize the opportunity for exposure in the lab. This includes the elimination of sharps for lab procedures by substitution with plastic and/or safe sharp alternatives. These practices should be included in the training program and evaluated during personnel competency assessments.

Step 5:

Protective Equipment – review of protective equipment that separates the user from the hazard:

The identification of protective equipment is a combination of**:**

- Personal protective clothing such as lab coats or gowns, gloves (double gloves for higher risk work), face protection (i.e., chin length face shield), safety glasses or goggles (depending on splash potential), and a mask.
- All engineering controls such as a biological safety cabinet (BSC), bench shields, sharps containers, vacuum system filters, biomedical waste containers, transport containers, etc. that will be used to place a barrier between the biohazard and the staff.

Step 6:

Places – review the location where the work will be conducted and biohazardous materials stored:

The final element of Risk Management is to review all the places where this research will be conducted, and biohazardous materials will be stored. Verify that all air flows into these laboratories and not out of them, 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 in that space.

Once again, after you've detailed your written risk assessment and have crafted biocontainment SOPs for the proposed research or work, please contact the EHS Biosafety Office or your assigned SA to schedule a walkthrough laboratory inspection to verify the suitability of the protective measures and the appropriateness of the proposed lab locations. The laboratory inspection report will be required by the IBC prior to their final review and approval of your protocol to use a biohazard or regulated biological material.

Consider the 6 Ps in each facet of laboratory work. Properly conducted, risk assessment can help prevent exposure to biohazards and minimize the potential for a laboratory-acquired infection. Remember that prior planning prevents poor performance.

After reading this section and relevant sections of the Biological Safety Manual, please contact the EHS Biosafety Office or your SA at 203-785-3550 for help applying the principles of risk assessment and risk management to experimental procedures.

6.1 Risk Assessment and Risk Management Table

A risk assessment/risk management matrix has been prepared to illustrate key elements of the process. Relevant sections providing additional details are indicated within the matrix.

Risk Assessment		Risk Management	
Pathogen	• Agent classification • 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 • CT DPH – infectious agents (IA) • USDA – restricted agents • CDC – select agents • FDA/NIH – human gene therapy	**Practices**	• Written set of SOPs with safety practices incorporated • Adherence to basic biosafety principles • Label labs, areas, and equipment housing BSL-2 or higher agents • Conduct lab inspections to review practices and containment equipment • Use trial experiments with non-infectious material to test new procedures/equipment
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 SOPs with safety practices incorporated	**Protective Equipment**	• 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 • Primary containment devices • Biological safety cabinets • Centrifuge safety buckets/rotors
Personnel	• Host immunity, immunization • Neoplastic disease • Infection, diabetes, Lupus • Immunosuppressive therapy • Age, race, sex, pregnancy • Surgery (splenectomy, gastrectomy) • Post-exposure prophylaxis • Attitude toward safety and comfort in lab • Open wounds, non-intact skin, eczema, dermatitis • Safety training and prior 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 Campus and Occupational Health • Investigation/review of incidents/spills, etc. to prevent future occurrence	**Place/ Laboratory**	• Risk Group/biosafety level (BSL) requirements • Aerosol risk • Restricted access • Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open • Labels • Containment laboratory with directional airflow

6.2 Routes of Exposures

In order for biological agents to cause disease, they must first enter or invade the body in sufficient numbers. Routes of entry include oral, respiratory, percutaneous, 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.

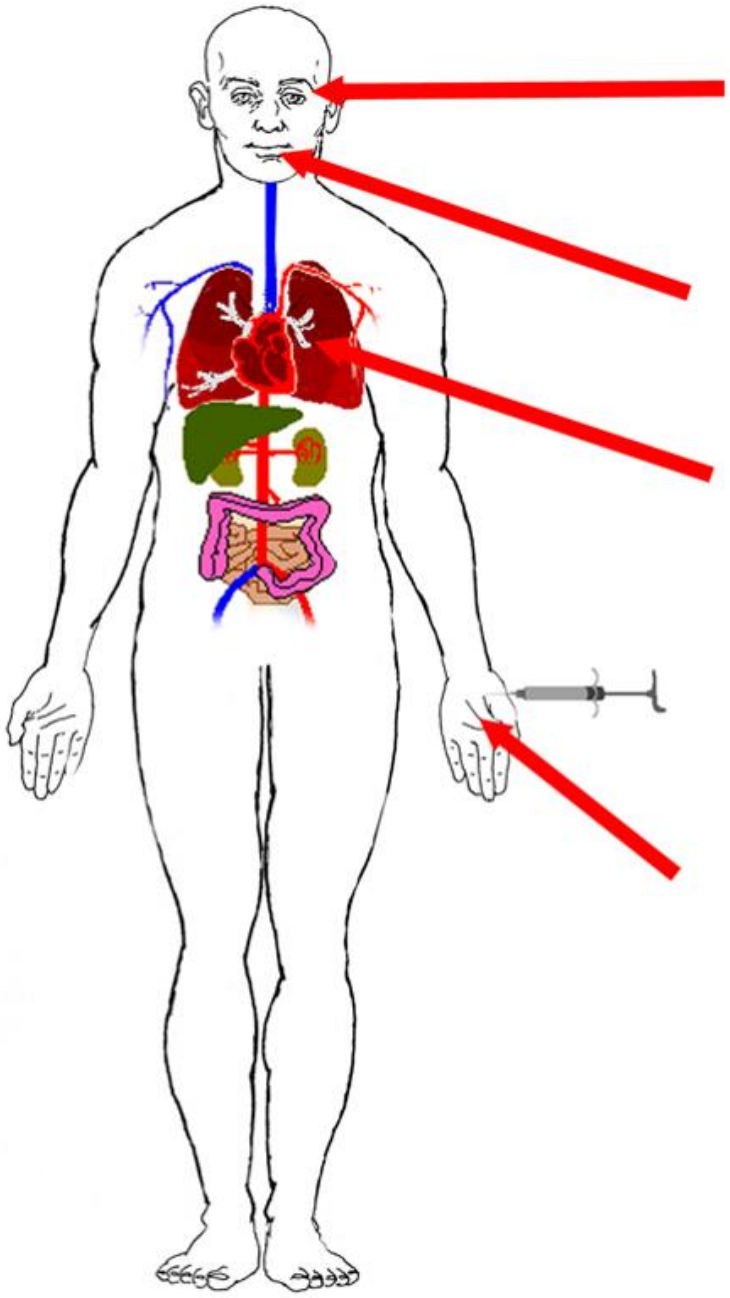
Other factors contribute to an individual's susceptibility to the disease process. These include age, immunological state, occupation, physical and geographic environment, and predisposing conditions, such as alcoholism and other drug abuse, pregnancy and diseases such as diabetes.

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 diagram and the table below for additional information on routes of exposure or contact the EHS Biosafety Office at 203-785-3550.

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 and 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.

Routes of Transmission for Infectious Agents (IA)



1 – Mucous Membranes: Exposures to mucous membranes of the eyes, nose, and mouth through splashes or splatters.

2 – Ingestion: Mouth pipetting, eating, drinking, or smoking in the lab.

3 – Inhalation: Breathing in respirable sized aerosols (<5µm) created during centrifuge leaks, spills, pipetting, etc.

4 – Percutaneous: Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds, including piercings and tattoos that have not healed yet, abrasions, and other skin wounds, such as eczema or other rashes.

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

Routes of Exposure and Corresponding Control Measures

Route of Transmission		Protection
Mucous Membranes	Exposures to mucous membranes of the eyes, nose, and mouth through splashes or splatters.	**Block Exposure to Mucous Membranes:** Use PPE such as a surgical mask and safety glasses or a full-face shield. Use engineering controls such as a biosafety cabinet (BSC) or a bench shield and follow good microbiological practices. Note: personal or prescription eyeglasses are not PPE. Use "over-the-glasses safety glasses" to protect your eyes when wearing personal or prescription eyeglasses in the lab.
Ingestion	Mouth pipetting, eating, drinking, or smoking in the lab.	**Follow Good Microbiological Practices:** Use mechanical pipettors, refrain from eating, drinking, use of oral nicotine products, and/or applying cosmetics in the lab. Always wash your hands after removing gloves and before exiting the lab.
Inhalation	Breathing in respirable sized aerosols (<5µm) created during centrifuge leaks, spills, pipetting, etc.	**Use Containment Equipment and Practices:** Utilize safety containment equipment including BSCs, sealed rotors or aerosol containment buckets for centrifuges, HEPA filtered respirator and follow good microbiological practices.
Percutaneous	Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds, including piercings and tattoos that have not healed yet, abrasions, and other skin wounds, such as eczema or other rashes.	**Use Extreme Precautions with Sharps:** Substitute plastic for glass whenever possible, dispose of sharps immediately after use in a rigid, leakproof needle box, use animal restraints and proper handling techniques, use cut resistant gloves when warranted. If you have a wound or abrasion, consult with your PI, cover it with waterproof bandages, and double glove. Always follow good microbiological practices.

Contact **(indirect transmission)**	Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e., benches, phones, computers, equipment handles) or by failure to wash hands after working.	**Follow Good Microbiological Practices:** Routinely decontaminate work surfaces. Use good personal hygiene, including avoiding touching your face and not applying cosmetics within the laboratory. Wash hands after removing gloves and before exiting the laboratory.

6.3 Biosafety levels

The CDC and NIH have established four biosafety levels (BSL) 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 BSLs of laboratory operation with selected agents infectious to humans. Also included in the BMBL is a parallel set of BSLs for research involving small laboratory animals. Only work at BSLs 1-3 is permitted at Yale University. No BSL 4 work is allowed at Yale University.

Below is a summary of practices, equipment and facility requirements for agents assigned to BSL 1–3.

6.3.1 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.	**Equipment:** None required. **PPE:** laboratory coats; gloves; safety glasses.	Laboratory doors; sink for handwashing; windows fitted with screens; adequate lighting.
2	Associated with human disease, hazards with potential for transmission by 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.	**Equipment:** 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; safety glasses; face or respiratory protection as needed.	**BSL-1 criteria plus:** • Self-closing and locking doors; • 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.	**Equipment:** 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 criteria plus:** • Physical separation from access corridors; • Self-closing, double- door access; • Hands-free sink; • Exhausted air not recirculated; • Negative airflow into laboratory; • Autoclave available, preferably within laboratory.

Adapted from BMBL 6th ed.

7 Signs and Labels

7.1 Signs

7.1.1 Door Signs/Laboratory Safety Information Cards

A Door Sign/Laboratory Safety Information card must be completed and posted at the entryway to all laboratories displaying the following information at a minimum:

- Biosafety level (BSL).
- The PI or other responsible personnel's name and telephone number.
- PPE requirements.
- Required procedures for entering and exiting the laboratory.

General occupational health requirements may be listed for specialized labs.

7.1.2 Biosafety Level

Entryways to research and clinical areas that handle biological materials must be posted with the appropriate BSL (BSL-1, BSL-2, BSL-2+, BSL-3) according to the research conducted in the room or space as approved by the EHS Biosafety Office and Yale IBC.

7.2 Universal Biohazard Sign

Entryways to research and clinical areas that handle potentially human infectious material must be posted with a BSL-2 biohazard sign and symbol. HIV and HBV research laboratories and production facilities, laboratories working with certain infectious agents (IA) that require special provisions for entry (e.g., medical consultation), and BSL-2, BSL-2+ and BSL-3 laboratories must have a biohazard door sign posted on all access doors.

The sign includes the universal biohazard symbol, bears the legend "Biohazard," any special entrance requirements, the name and phone numbers of the Principal Investigator (PI) or any other responsible persons, and may identify the name of the IA. The following elements are included on the door sign:

(Name of IA)
(Special entrance requirements)
(Name, telephone number of the PI or other responsible person)

The door signs shall include a biohazard label that is fluorescent orange or orange-red (or predominantly so) with lettering or symbols in a contrasting color.

7.3 Labels and Color-Coding of Biohazard Labels

Inside the facility, warning labels shall be affixed to containers of medical waste, refrigerators, freezers, incubators, centrifuges and any other equipment that potentially contains BSL-2 or BSL-3 agents, human blood or "other potentially infectious material".

Warning labels shall also be affixed to other containers used to store, transport or ship BSL-2 or BSL-3 agents, human blood or "other potentially infectious material". **Note: Shipping blood and "other potentially infectious material" not suspected of harboring an IAs may not require the biohazard warning label, only an Exempt Human Specimen or Exempt Animal Specimen label**. Labels must have the universal biohazard symbol and bear the legend "Biohazard" (see figure on below).

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

7.4 Labeling Equipment Sent Out for Repair or Disposal

Contaminated and potentially contaminated equipment sent out for repair or disposal must be decontaminated as thoroughly as possible. Affix a tag to the equipment indicating when the equipment was decontaminated, what disinfectant was used, and the name of the person who performed the decontamination. Thorough decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. Decontaminate the equipment to the degree possible (flushing lines or wiping down the exterior) and affix a label to the equipment before sending it out for repair. The label must convey this information to all affected workers (service representatives, manufacturer, etc.). Equipment tags can be obtained by contacting Yale Environmental Health and Safety at 203-785-3550 or using this link.

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.

8 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.

8.1 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 a 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 (PIs) 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 tends to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.
- Working at a faster than normal rate of speed is a significant causal factor in laboratory accidents.
- Failure to follow approved SOPs and policies is a frequent cause of accidents. These deviations can lead to increased risk.
- Accidents occur most frequently during routine procedures. Currently, the most frequently reported laboratory accidents involve sharps exposures in animal research or clinical settings.
- 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.
- Comfort performing laboratory procedures is essential. Researchers should be properly trained before performing procedures involving biohazards. A graded approach (working with lower risk materials prior to moving on to higher risk hazards) should be implemented if a researcher is unfamiliar with a procedure they will be performing.

8.2 Biosafety Level 1

These laboratory practices are expected to be performed at BSL-1, including ABSL-1 facilities. Adhering to these basic principles sets a foundation for good laboratory practices in these and higher biosafety level (BSL) laboratories.

- Keep laboratory door closed at all times.
- Keep animals and plants not used in BSL-1 experiments out of the laboratory.
- Do not smoke, eat, drink, or store food in BSL-1 areas.
- Wear eye protection, gloves, and laboratory coats or gowns when appropriate.
- Tie back long hair so that it cannot contact hands, specimens, containers, or equipment.
- Do not mouth pipette. Use mechanical pipetting devices.
- Use procedures that minimize the generation of aerosols.
- Minimize the use of sharps whenever possible. Use plastic alternatives to glass and select sharps devices with built-in safety features.
- Disinfect work surfaces daily and immediately after a spill.
- Decontaminate all biological waste before discarding it. 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 contamination.
- Keep areas neat and clean.
- Wash hands after completing experimental procedures and before leaving the laboratory.
- Remove PPE prior to leaving the laboratory space.

8.3 Biosafety Level 2 (BSL-2)

These practices are expected to be performed in laboratories designated as BSL-2. Additionally, for laboratories that are registered for work with human pathogens, these practices are required to meet the expectations of the Connecticut Department of Public Health (CT DPH).

- Keep laboratory door closed at all times.
- Post a universal biohazard label on equipment where biohazardous materials are used/stored.
- Allow only people informed of the research to enter BSL-2 areas.
- Keep animals and plants not used in experiments out of the laboratory.
- Do not smoke, use oral nicotine products, eat, drink, or store food in laboratory areas.
- Wear eye protection, gloves, and laboratory coats or gowns when appropriate.
- Tie back long hair so that it cannot contact hands, specimens, containers, or equipment.
- Do not mouth pipette. Use mechanical pipetting devices.
- Use procedures that minimize the generation of aerosols.
- Minimize the use of sharps whenever possible. Use plastic alternatives to glass and select sharps devices with built-in safety features.
- Use biological safety cabinets (BSC) to contain aerosol-producing equipment.
- Disinfect work surfaces daily and immediately after a spill.
- Decontaminate all biological waste before discarding it. 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 contamination.
- Disinfect work surfaces daily and immediately after a spill.
- Keep areas neat and clean.
- Wash hands after completing experimental procedures and before leaving the laboratory.
- Remove PPE prior to leaving the laboratory space.

8.4 Biosafety Level 2 Enhanced (BSL-2+)

BSL-2+ is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BSL-2 procedures. Generally, BSL-3 practices are mandated in a space designed for BSL-2 work. It is preferred that the BSL-2 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 contacts, and entry requirements is posted on the door while BSL-2+ 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 BSL-2 or BSL-1. Some laboratories at Yale are designed to be BSL-2+ at all times.

All manipulations of BSL-2+ material are conducted in a Class II BSC and secondary containment is utilized for centrifugation and other potential aerosol generating procedures.

Review the BSL-2+ Work Practices SOP for more information. The “Biosafety Work Practices for Cell Culture: BSL2 and BSL2-Enhanced-Web-Based" course can be accessed via Workday Learning. Please consult the Biosafety Office prior to initiating any work at BSL-2+.

Detailed information on BSL-3 work practices is provided in the Yale Biological Safety Level 3 (BSL-3) manual. The BSL-3 Manual is available on the Yale EHS website under Biosafety Policies and Procedures or at this link.

8.5 Cell Culture

When performing cell culture experiments, personnel are required to follow the procedures outlined in this section. Not only do these procedures protect personnel and the environment from potential contamination, but they also aim to protect the integrity of the culture.

- Wear long sleeved gowns / laboratory coats with knit cuffs and examination gloves when working in the BSC.
- PPE and other contaminated items should be disinfected or autoclaved before being laundered, reused, or disposed of.
- Glassware should be thoroughly cleaned and rinsed, by washing repeatedly with tap water and distilled water.
- Both liquid and consumables cell culture waste must be decontaminated before being disposed of properly. Discard empty tubes immediately.
- Maintain a clean lab coat reserved solely for cell culture work. Never take used PPE home to launder.
- Avoid talking during culture manipulations as aerosols may be drawn into the work area.
- Keep open tubes parallel to the airflow.
- After transferring inoculum, always recap vials or tubes.
- Always pipet biohazardous materials down sides of tubes or flasks to reduce aerosol generation.
- Close tip boxes between use to prevent contamination.
- Place tubes in a rack and not on the work surface.
- Work with one specimen at a time; recap the tube before going to the next.
- Only bring what is needed for the experiment into the BSC to avoid any clutter that can block airflow.

If a problem with contamination develops, please call the Biosafety Office at 203-785-3550 for further assistance.

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

Please refer to the Yale University Shipping Policy for information regarding shipping of hazardous materials. Additionally, refer to section 9 of this manual for transport of biohazardous materials between laboratories.

8.7 Basic Microbiological Practices

8.7.1 Culture Plates, Tubes and Bottles

Particular care is required when opening plates, tubes, or bottles containing biohazards, as this may release aerosols. Such cultures must be manipulated in a BSC.

Agitation of samples such as centrifuging, vortexing, homogenizing, shaking, and swirling creates aerosols. When a liquid culture is re-suspended, a few minutes should elapse prior to opening the container to allow any aerosols created to settle. As a reminder, perform work with pathogens inside a BSC.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. When using reusable loops, use ones that are fully anchored to the stem as they create less aerosols. Aerosols can also be minimized by allowing the loop 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 sterile inoculating loops or spreaders are available commercially. Rather than decontaminating them immediately after using them with heat, they can be placed in a solid biomedical waste container for decontamination.

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 BSC.

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 BSC 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 or lyophilized hazardous materials should be opened in a BSC. A dropped vial of lyophilized material can generate up to 4 times the amount of aerosols than a similar spill of a liquid culture. See the following reference for specific details.

Kenny MT, Sabel FL. 1968. Particle Size Distribution of *Serratia marcescens* Aerosols Created During Common Laboratory Procedures and Simulated Laboratory Accidents. Appl Microbiol 16: https://doi.org/10.1128/am.16.8.1146-1150.1968

When using a shaker for cultures containing pathogens, please use filtered closures to prevent aerosol release instead of aluminum foil or other closures. Please see the EHS Safety Products document on the EHS Biological Safety Tools and Resources webpage for more information regarding filtered flask closures.

8.7.2 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 must be done in BSCs or a

chemical fume hood if working with a toxin. 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 BSC and wear gloves and use tools such as an ampoule opener. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling and withdraw the contents into a fresh container. Some researchers may desire to use commercially available ampoules prescored for easy opening. However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, 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. After decontamination, discard ampoule(s) in sharps container or plastic solid walled biohazard waste container.

8.7.3 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 BSC. A suitable disinfectant should be at hand and used frequently. Please contact the Biosafety Office at 203-785-3550 prior to initiating any egg cultures with pathogens to ensure proper SOPs.

8.7.4 Sources of Contamination

If contamination is experienced in the laboratory, the following items may be sources of contamination. For additional assistance, please contact Environmental Health and Safety (EHS) at 203-785-3550.

Microbial contamination in a lab can stem from various sources, including **airborne particles, personnel, sample cross-contamination, and even equipment or instrument contamination**. Poor laboratory techniques, improper handling of reagents and samples, and inadequate cleaning procedures also contribute to contamination.

Here's a more detailed breakdown:

1. Airborne Particles:

Bacteria, molds, and spores present in the air can settle onto surfaces and contaminate samples. Inadequate ventilation or high bacterial loads in the lab environment can increase the risk of airborne contamination.

2. Personnel:

Laboratory personnel can inadvertently introduce contaminants through direct contact with samples, equipment, or surfaces. Poor hand hygiene or improper use of personal protective equipment (PPE) can also contribute to contamination.

3. Sample Cross-Contamination:

Cross-contamination can occur when samples are improperly handled or stored, allowing microorganisms to transfer between different samples. Using contaminated equipment or reagents can also lead to cross-contamination.

4. Equipment and Instrument Contamination:

Equipment, such as pipettes, culture flasks, and incubators, can harbor microorganisms if not properly cleaned or sterilized. Reusable labware should be rinsed thoroughly and air-dried, as autoclaving may not eliminate detergent residue.

5. Other Sources:

Water used for preparing media or buffers can be a source of contamination.
Raw materials used in the lab, such as reagents and chemicals, can carry high levels of bioburden.
Improper procedures, such as using unplugged pipettes or not following sterile techniques, can increase the risk of contamination.

Personal items, such as coats, hats, umbrellas, purses, etc., do not belong in the laboratory. These articles should be stored elsewhere, such as an office.

Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens, laboratory supplies, and outside air is a complication that may affect or invalidate the results of an experiment.

Personal Work Practices

In the laboratory, personal work practices prioritize safety and adherence to procedures to protect individuals and maintain data integrity. This includes wearing appropriate PPE, maintaining a clean and organized workspace, following established safety protocols, and engaging in good hygiene practices.

1. Personal Protective Equipment (PPE):

- Lab coats, gloves, eye protection (safety glasses or goggles), and closed-toe shoes are essential for protecting against hazards.
- Specific PPE requirements vary based on the nature of the work and hazards present.

2. Good Hygiene Practices:

- Avoid eating, drinking, smoking, or applying cosmetics in the lab.
- Wash hands thoroughly after removing gloves and before leaving the lab.
- Do not handle contact lenses or touch common objects like phones or doorknobs while wearing gloves.

3. Safe Work Practices:

- Always follow established Standard Operating Procedures (SOPs).
- Never pipette by mouth.
- Keep the lab clean and uncluttered.
- Properly label all chemical containers.
- Store chemicals properly and ensure they are not left open.
- Always wash hands after contact with chemicals or other hazardous materials.

4. Housekeeping:

- Keep work areas clean and free from clutter.
- Regularly clean up spills and wipe down benches.
- Ensure aisles and passageways are clear and not blocked.

- Shaving and brushing 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 the mouth, nose, eyes, face, and hair. This may prevent self-inoculation.
- For product protection, a person with long hair should wear a suitable hair net or head cover that can be decontaminated. Hair may be tied back. 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 flames 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 personnel exposed to caustic chemicals unless safety glasses with side shields, goggles or a 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.
- When employees are subject to potential occupational infection, the shower and/or face/hand-washing facilities should be provided with germicidal soap.
- 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 the 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. The 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.
- 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 waterproof bandages and double gloving.

8.8 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. A well-conceived and well executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and

provides an area that promotes the efficient use of decontaminates in the event of inadvertent release of an etiologic agent.

Housekeeping and cleaning are also required in the three most prominent laboratory regulations, standards and guidelines in the United States: the OSHA Bloodborne Pathogens Standard (1910.1030); the NIH Guidelines; and the BMBL. These regulatory documents note the requirement for cleaning schedules, maintaining clean and neat labs, and keeping lab areas accessible for cleaning.

8.8.1 Objectives of Housekeeping

The objectives of housekeeping in the laboratory are to:

- Provide an orderly work area conducive to the research.
- Provide work areas devoid of physical hazards.
- Provide a clean work area to limit contamination.
- Prevent the spread of contamination by damp mopping instead of sweeping floors.
- Prevent the accumulation of materials from current and past experiments that can create a hazard or clutter the work environment.

In regard to preventing the accumulation of research materials, Yale EHS has created a series of tools for the inventory of cold storage units on campus and these can be accessed on the EHS Biological Safety Tools and Resources webpage under High Risk Biomaterial Laboratory Inventory Template, High Risk Biomaterial Shared Freezer Storage Map Template, and High Risk Biomaterial Storage Inventory Template.

EHS has also created a researcher exit interview checklist that can be utilized to identify samples marked for retention by the lab, or those that can be discarded to minimize clutter within cold storage devices. This form is also accessible on the Yale EHS webpage here.

For general laboratories at the University, Yale Facilities will perform basic housekeeping for laboratories, such as emptying trash and cleaning lab floors. Please note that if you work in a biohazard containment laboratory or other specialized biohazard facility, your staff is responsible for the daily housekeeping chores.

Benefits of proper housekeeping and maintaining neat and clean laboratories include:

- Minimizing the build-up of dust and debris which could lead to contamination events in the lab.
- Facilitating regular cleaning by keeping areas on floors and benches accessible.
- Preventing the interference with disinfection by minimizing dust and debris on work areas that could harbor organic materials that could inhibit halogen based and other disinfectants.
- Supporting an orderly lab environment where critical items such as lab coats and other PPE have a consistent storage place to promote removal prior to leaving the lab.
- Supporting the safe conduct of research in your laboratory areas.
- Protecting those with allergies to dust mites and animal dander by keeping their exposures to a minimal level.

Safety Moment: Remove your gloves before heading out of labs or animal areas for public spaces!

A serious medical issue was traced back to individuals wearing their PPE, lab coats and gloves in and out of laboratories. An occupant of a research building with an animal facility in the basement experienced an anaphylactic event that required a 911 call and emergency medical response. The researcher had an allergy to rodent urinary proteins and had stopped working with animals. When she arrived the morning of the incident, she touched the door handle into her lab space and experienced the reaction. EHS investigation after the incident identified numerous cases of individuals coming up in the early morning from the animal facility and touching doorknobs with gloves that were worn while

working directly with animals. Personal protective equipment (PPE) must be removed before leaving animal facilities and laboratories, and this extends to gloves also.

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 animal care facilities.

8.8.2 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. Schedules must be created and adhered to in order to ensure that dust and debris do not build up. Wet mopping is preferred over dry sweeping to minimize the spread of dust throughout the lab. If vacuuming is necessary, a vacuum cleaner with a HEPA filter should be utilized.

When establishing cleaning schedules, the following equipment and areas merit consideration for regular cleaning and inspection.

- Aisles
- Benchtops
- 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

8.8.3 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 about the research environment. The recommended approach to housekeeping is to assign housekeeping tasks to individuals for their immediate work areas and for teams to cooperate on housekeeping tasks for shared spaces. 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 to be accomplished. The supervisor should provide schedules and perform frequent inspection to assure compliance.

Additional detailed references on housekeeping and laboratory cleaning can be obtained in the three regulations, standards and guidelines listed above, and in the 1979 Laboratory Safety Monograph, which can be accessed at this link.

9 Personal Protective Equipment (PPE)

Multidisciplinary research conducted in Yale University laboratories requires that PPE, including protective clothing and safety apparatus or equipment, be used to protect the researcher from contact with infectious, toxic and corrosive agents, excessive heat, cold, fire, and other physical hazards. Suitable 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 it serves as the final line of defense. Good laboratory techniques, procedures, and appropriate laboratory equipment and engineering controls are the primary barriers against potential exposure to hazardous agents and harmful elements. For additional information you are urged to consult the Biosafety Office.

9.1 Laboratory PPE

PPE represents a range of protective items used in laboratories to protect researchers from exposures. PPE may be reusable or disposable. Whichever is used, it must be durable, designed to provide protection, and prevent exposure of the skin, mucous membranes, and personal clothing to harmful agents, as well as be compatible with any chemicals used in the lab.

Laboratory PPE and proper donning and doffing practices protect the individual, the experiment, and prevents contamination from leaving the lab. If proper precautions are not taken, contaminated PPE may carry infectious materials outside the laboratory and into other work areas, cafeterias, or the home. Infectious agents (IA) or other contaminants can remain viable on cotton and wool fabrics and be disseminated from these fabrics.

Some additional points:

- All individuals entering the laboratory must wear sensible clothing that covers and protects your legs down to your ankles and shoes must completely cover the foot.
- Provisions should be made for PPE to be provided to visitors and maintenance or security personnel where required.
- PPE worn within the laboratory must 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.

9.1.1 Gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired

level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. Quality assurance is an important consideration.

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

The most common gloves used in the laboratory are disposable exam gloves. Disposable (single use) gloves provide a barrier between IA and the skin. Gloves are generally nitrile, latex, vinyl or polyvinyl chloride. Because of the concerns over developing or exacerbating allergies, latex gloves are not used as much, especially powdered latex gloves, which could spread latex allergens through the laboratory if used. Vinyl gloves are not as sturdy and offer less resistance to standard lab chemicals than other types, leaving nitrile gloves as the most often used in laboratories today. As no single glove is impervious to all chemicals, please evaluate the chemicals used for protection against those chemicals before use. You may also contact Yale EHS for information on chemical permeability through lab gloves.

Glove use is a basic precept of preventing IA transmission. It is important to be aware that lab exam gloves are not 100% leak proof. The Food and Drug Administration (FDA) allows manufacturers to produce gloves with an overall leak rate of 2.5%. That means that 1 of every 40 gloves will have a hole in it, that you may not be able to see, that would allow biohazards to get through. In other words, for every 20 pairs of gloves you will wear in your career, one glove of the pair will likely have a leak before use. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common. Because of the risk associated with gloves that leak and breaks in the skin, double gloving has been utilized when working with biohazards to greatly increase worker protection. Gloves will also acquire more leaks with use, especially around the knuckles or other stretch points. Leak rates on gloves removed from surgeons and nurses in the operating room show leak rates of 15% after 30 minutes of use after removal and testing. Due to these studies, it is important to change gloves roughly every 30 minutes when working with biohazards.

Gloves shall be removed and hands washed before exiting the laboratory. Do not wear gloves outside of the laboratory, in hallways, elevators, break rooms and other non-lab locations. If you must transport biological materials outside of the laboratory between floors or buildings, use a sealed secondary transport container. Select a transport container that is unbreakable and leak proof. Used a sealed tube, such as a gasketed screw-capped plastic vial, and place paper towels inside the transport container as absorbent. A biohazard label with the identity of the contents and the lab's contact phone number on the label. PPE, such as gloves and a lab coat are not needed for transport. Bring gloves with you to put them on once at the next lab location or keep gloves available in this second work area.

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.

Cut-resistant and puncture resistant gloves have also been utilized as additional protection when working with sharps and animals. Cut-resistant gloves are useful when using equipment such as microtomes and cryostats, or when performing necropsies that involve scalpels. Cut-resistant gloves offer protection against slicing injuries or lacerations; they will not prevent needle sticks or punctures. EHS recommends utilizing gloves rated by the American National Standards Institute (ANSI) level 4 or higher. Cut-resistant gloves are worn below lab gloves and do take some time for acclimatization or getting used to. Puncture resistant gloves help protect against needlesticks and can be utilized when working with needles and syringes, fine tipped forceps and small scissors. They also offer protection against bites from small animals such as mice or rats. Much thicker puncture-resistant gloves, such as gauntlet leather, chain mail, or thick polymer fabrics, can be utilized when working with larger research animals, such as non-human primates (NHP).

These are generally worn over lab exam gloves. Cut or puncture resistant gloves can be worn on both hands or on the non-dominant hand, depending on the potential risks of exposure.

Considerations for the selection and use of gloves and reminders:

- Gloves are not 100% leakproof; change gloves periodically and when soiled and always wash hands after removing gloves or other PPE.
- Glove permeability will reduce with time and use of the glove. Be prepared to change gloves periodically, at least every 30 minutes.
- Double gloving offers much more protection when working with human pathogens and higher risk biohazards.
- Gloves will not prevent needle sticks or other puncture injuries.
- Wear cut-resistant gloves when working with scalpels, cryostats and microtomes.
- Wear puncture resistant gloves from protection against animal bites, and punctures with needles and other sharps.
- Check gloves for visible tears before use.
- Avoid wetting latex examination gloves as water or disinfectants will encourage wicking and leaking in the latex membrane which can lead to increased permeation.
- Do not reuse examination gloves; discard contaminated gloves in a biohazard bag immediately after use.
- Change gloves as soon as feasible following contamination with biohazards in the lab.
- Double glove or use household utility gloves when cleaning spills. Household utility gloves may be decontaminated and reused (replace when compromised).

9.1.2 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.

9.1.3 Sleeve Covers

- Disposable sleeve covers have been used in labs working with biohazards to protect the wrist, forearm, inside the lab coat sleeve, watches, bracelets and other jewelry from contamination when working in the lab. They also have been deployed to preserve the life of sturdy disposable back-fastening gowns, by protecting the arms of the disposable gown, especially when working inside the biosafety cabinet (BSC).
- Factors for consideration for wrist protection. Standard open-cuff lab coats do not protect the wrist because there is a wide opening. When working the open part of the lab coat opening will pull away from the gloves, exposing the wrist and any jewelry worn. If working inside a BSC, the same configuration of the open cuff lab coat and exam gloves can create significant opportunities for personal contamination. The smoke-split of the BSC pushes ½ the air inside the BSC towards both the front and rear of the BSC while it is in operation. As you work inside the BSC, aerosols are generated from standard laboratory procedures such as pipetting. These aerosols are moved toward the front opening of the BSC and then down into the front grilles at the opening of the cabinet. If wearing an open cuff lab coat and normal exam gloves, these aerosols will contaminate the open skin of the wrist, the forearms, any jewelry, and inside and outside of the lab coat sleeve. Sleeve covers work to protect the lab coat from contamination, the wrists from exposure, and prevent having to extend hand washing to the wrist and forearms. Longer

sleeved gloves, lab coats with knit-grip cuffs, and even elastics to hold open cuff lab coats are effective at protecting the wrists from contamination.

9.1.4 Shoes

Shoes worn in the laboratory must be solid covered and closed toe. Protective shoes are required for certain work activities. When working with IAs it is advisable to wear shoe covers in higher risk laboratories, which can be decontaminated (autoclaved) before disposal, over street shoes. Some cell culture laboratories have recommended a change from street shoes to specific laboratory shoes for protection of cultures from contamination.

In certain animal facilities the Yale Animal Resources Center (YARC) requires personnel to wear overshoes (shoe covers or booties) to protect the animals in containment areas. Similarly, people who work with animals and perform cage washing are required to wear safety, slip-resistant, and protective shoes. All personnel working under YARC must follow these and other requirements of YARC.

9.1.5 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. Solid front or back-fastening gowns are worn in high-risk biohazard laboratories. Lab coats are worn in BSL-1 and BSL-2 laboratories. Lab coats protect your personal clothing and skin from contamination. Although lab coats are not 100% leakproof, they offer a protective barrier that provides time to remove the garment before permeation to skin and clothing. 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 must be implemented to ensure the clothing is buttoned and 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.

9.1.6 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.
- Face shields, or the use of safety glasses and a surgical mask, protect the eyes, nose and mouth from contamination from splashes or splatter.
- Face protection also can serve as a stop sign to help remind researchers to avoid touching their face or facial mucous membranes while working.
- 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.

Safety Moment: Avoid Touching Your Face

- In a landmark study by researchers at the University of Utah, the authors who were observing clinical lab researchers work with biohazards known to be transmitted via the eyes, nose, or mouth, identified the following information.
- 72% of all researchers observed touched their face at least once during the study. Those touching their eyes, nose or mouth were most concerning.
- On average, they observed 2.6 hand-to-face contacts per hour.
- Only 3 of the 93 researchers observed had any eye or face protection (safety glasses, goggles, or face shield).
- Most did not wear anything on their face. Notably, from their observations, they noted that from the procedures conducted, there was a very high likelihood that the researchers' fingers, hands, and wrists were likely contaminated with the pathogens that they were handling. They also stressed that hand-to-face contact is a likely contributor to the 80% of laboratory acquired infections that have been documented but those infected could not identify a specific route of exposure.
- Adding credence to this study is that the fact that researchers will report exposures that they can "feel", such as needlesticks, other lacerations, or splashes of biohazards to the facial mucous membranes. But they won't report what they can't feel, exposure to biohazardous aerosols, accidental ingestion, or forgetting that they touched their face, or had very minor breaks in their skin under leaking gloves. More information regarding this study can be found in their reference on this topic below.
- Johnston, J.D., et al, "The Influence of Risk Perception on Biosafety Level-2 Laboratory Workers' Hand-To-Face Contact Behaviors," Journal of Occupational and Environmental Hygiene, Vol. 11, pp 625-632, September 2014.

9.2 Respiratory Protection

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to 10 organisms, when inhaled, may cause infection. Particles with an effective aerodynamic diameter of between 0.5 and

5.0 mm (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 (BSC), should 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 EHS. 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 EHS is notified whenever the use of a respirator is being considered. The responsible individuals in EHS can assist in evaluating the procedure, selecting the proper respirator, and provide the required training and fit testing. The Campus and Occupational Health Office must also be notified so that medical surveillance and clearance can be issued prior to wearing the respirator.

Additional information can be found on Yale University's Respiratory Protection Program webpage.

Safety Moment: Aerosols

Why is awareness of aerosols critical when working with biohazards?

Aerosols are solid or liquid particles suspended in air. In laboratories they are created anytime energy is imparted to a culture. During the COVID-19 pandemic everyone learned a great deal about aerosols as a factor of how the coronavirus is transmitted. There are many factors that impact aerosol formation, spread, survival and transmission. We'll focus on a few simple ones, size and weight. Larger aerosols will settle or fall out of the air relatively quickly after they are created. Think of "bowling balls." These heavier particles present a risk of surface contamination and cause exposure by directly contacting facial mucous membranes, and open wounds. By contaminating surfaces and items, they present a risk of self-inoculation if touched by a researcher before these surfaces are disinfected. Smaller aerosols (think feathers and very tiny specs of dust) are the ones that are a more significant problem if generated in a laboratory. Very small aerosols have the potential to remain suspended in the air of the lab where they have been created or they can move to other places on air currents, sometimes far away from the location where they were they were generated. The small invisible microscopic sized aerosols pose a greater risk of airborne transmission. Up to 80% of the published laboratory acquired infections have an unknown route of exposure. Aerosols are likely a contributing factor in many of these infections.

Early biosafety pioneers identified that labs can be more dangerous than nature, due to the amplification of pathogens in research and the repeating of experiments over and over. Lab spaces are also confined

when compared to the massive ventilation of outdoor areas. They also identified that you can have unnatural routes of exposure in the laboratory. These pioneers were also in agreement that in the laboratory environment, aerosols must be confined as close as possible to their point of generation. The first two laboratory acquired infections with rabies virus were transmitted by aerosols created outside of primary containment. It is likely that the aerosol generating procedure (homogenization of rabid goat brains that were not sufficiently fixed in a standard kitchen blender that lacked containment) created airborne viral particles that were inhaled by the researchers. These aerosols likely had a direct avenue to the brain of the researchers through cranial nerves (olfactory and trigeminal) located in the nasal cavity.

What laboratory procedures create aerosols?

All of them! All lab procedures have the potential to create aerosols. Factors to consider are the amount of energy imparted, the volume of liquids and the length of the laboratory procedure (e.g. the amount of individual or repeated manipulations). Here is a brief look at some of the procedures that have been linked to aerosol creation and risk of infection in the laboratory.

Centrifugation

The high speeds and revolutions associated with centrifugation presents a significant risk of aerosol dissemination and risk of exposure to researchers. The use of open tubes, cracked tubes, snap-cap tubes that are not sealed, inappropriate or expired tubes, overfilled tubes, not using secondary containment or overfilling tubes are examples of risk factors when centrifuging biohazards. Most centrifuge manufacturers have designed leak-proof secondary containment safety buckets or have gasketed sealed rotors to confine aerosols if they were created from a leak during a centrifuge run. These safety buckets and sealed rotors are then designed to be opened inside a BSC which would further contain any aerosols if there had been a leak inside the centrifuge during a run with biohazards. Porton Down labs in the UK is the leading test agency for containment of centrifuges. They utilize a protocol that involves a cracked tube and then examines if the secondary containment buckets or rotors will contain the leak. Look for Porton Down certification of your safety bucket or sealed rotor when purchasing new centrifuges. You can also call the EHS Biosafety Office for assistance with your centrifuge containment questions.

Pipetting

Pipetting is done day in and day out in microbiological and biomedical laboratories. Most performing these procedures are unaware that pipetting also can create aerosols, especially if laboratorians are working too quickly and not carefully. Dr. Mark Chatigny counted the aerosols generated by researchers at the NIH in one of his studies and the results were eye-opening. See the table below to look at the risk that pipetting biohazards may represent in your laboratory. Use a BSC to confine aerosols when pipetting them.

Table of Recovery of Aerosols from Pipetting

Cell sorting

High speed cell sorters use very high pressures to create a single stream of individual cells for separating specifically labeled cells for research purposes. Some of these units can represent a significant risk of aerosol formation if there is a clog or deflection during a sort.

The following video demonstrates how significant the aerosol formation can be. Companies that make cell sorters have built in containment features and have worked with other companies to develop enclosures that help confine aerosols within the cell sorter when in use. The International Society for Advancement in Cytometry (ISAC) also has a lot of biosafety information for researchers regarding containment when sorting biohazards.

Spills

Kenny and Sabel, in their 1968 study where they dropped a flask of culture then measured the aerosols created by this action, led biosafety officers to create emergency spill response protocols to have everyone immediately evacuate the laboratory in the event of a spill or release of biohazards outside of primary containment. Alan Bennet of the UK has also studied biohazard aerosols in a variety of different spill scenarios to continue to demonstrate the risks that spills present to those.

Kenney and Sabel, 1968

If a spill occurs involving a biohazard in your laboratory, please get everyone out and notify Yale EHS of the incident on the EHS emergency line at 203-785-3555.

Homogenizing, vortexing, blending

There are many other equipment risks for aerosols in the laboratory. Due to the potential energy imparted, these three procedures may also represent a significant risk. Wherever possible, these procedures should be performed within a BSC. Contact the Yale EHS Biosafety Office for more information on containment for homogenizers or blenders if needed for use with biohazards.

How do we control or confine aerosols? Performing all procedures that generate aerosols inside a BSC is the primary way that we can work safely in the laboratory. If procedures are performed on the bench, practices must be performed very carefully and meticulously to minimize aerosol formation and spread. Please work with the Yale EHS Biosafety Office to have your procedures evaluated before working with biohazards on the bench.

9.3 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 (i.e., 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 IAs or handling spills.
- Thicker household utility gloves can be worn for cleaning blood or BSL-2 spills.
- Utility gloves can be decontaminated and reused until the integrity of the glove is compromised. Disposable gloves should never be washed or reused.
- 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 BSC. 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, including fresh tattoos and/or piercings. Utilize this poster for a visual aid reminder in your laboratory.

- Avoid working with RG2, RG3, or other potentially infectious materials (OPIM) 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 IA 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 in biohazardous areas, 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, 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 PAPR) 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.

9.4 PPE Requirements Table

PPE	Biosafety Level 1	Biosafety Level 2	Biosafety Level 3
Gloves	Required by Yale PPE Policy for protection in labs. Check your lab's PPE Assessment. Recommended nationally to prevent skin or clothing contact with BSL-1 materials. Note: work that may involve radioactive materials or chemicals may require the use of additional personal protective clothing.	Required.	Double gloving is required.
Lab Coat	Required by Yale PPE Policy for protection in labs. Check your lab's PPE Assessment. Lab coats prevent skin or clothing contact with BSL-1 materials. Note: work that may involve radioactive materials or chemicals may require the use of additional protective clothing.	Required.	Solid front protective clothing such as back fastening gown with tight fitting cuffs must be worn to protect clothing and skin from contact with IAs.
Face Protection	Required by Yale PPE Policy for protection in labs. Check your lab's PPE Assessment.	Required by Yale PPE Policy for protection in labs. Check your lab's PPE Assessment. 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 required in BSL-3 labs. This can be achieved by wearing a PAPR hood or face shield when utilizing an N95.

Respiratory Protection	Based on assessment by Yale Campus and Occupational Health, if necessary.	The use of respiratory protective equipment such as a PAPR or N95 may be recommended or required by the IBC and/or EHS on a case-by-case basis.	The use of respiratory protective equipment such as a PAPR will be recommended or required by the IBC and/or EHS on a case-by-case basis. The use of PAPRs is required for response and cleanup of a BSL-3 spill. All those who may wear a respirator must be enrolled in the EHS Respiratory Protection Program.
Other	N/A.	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.

10 Laboratory Equipment

10.1 Biological Safety Cabinets

Environmental Health and Safety (EHS) has a program that monitors the performance of biological safety cabinets (BSC) 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.

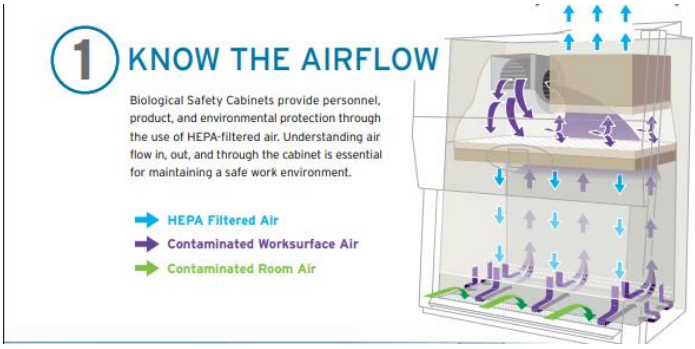
BSCs, when used properly, provide a clean work environment for research or patient care activities. BSCs offer personnel, product, and environmental protection. The BSC provides primary containment for infectious materials. The efficacy of BSCs depends upon the behavior of the operator and the orientation of the unit in the facility. It is essential to remember that only one person may perform work in a BSC at any given time. Two (or more) people working at a BSC is not permitted at Yale.

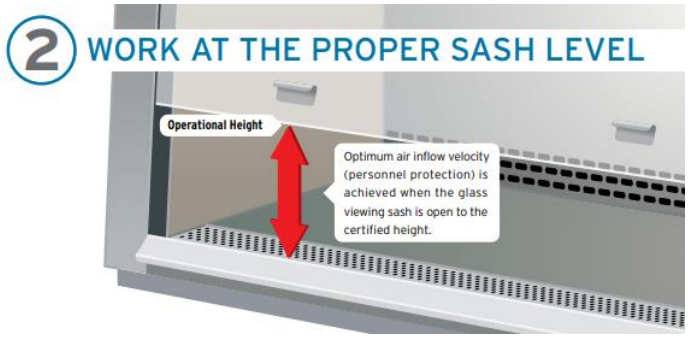
The BSC isolates biohazards from personnel by confining the biohazardous material in the unit. The BSC removes aerosolized biohazardous material by moving air through high efficiency particulate air (HEPA) filters. The intake air is filtered through a HEPA filter before entering the BSC work area. Exhaust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

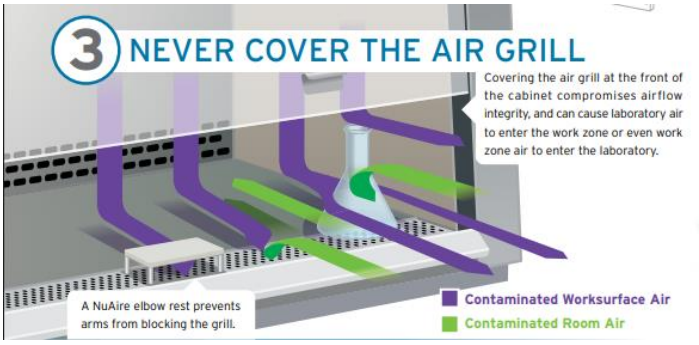
Operating Procedures for Class II Biological Safety Cabinet:

Before Use:

- If used, turn UV light off.
- Turn on fluorescent light and blower and allow BSC to purge for 2-3 minutes.
- Check the certification date (within 12 months) and ensure the drain valve is closed.
- Ensure the sash is at the correct height and there are no alarms.
- Disinfect all interior surfaces with 70% ethanol, 1-10% bleach or other suitable disinfectant.
- Place items required for procedure into cabinet. **Never obstruct the grilles.**

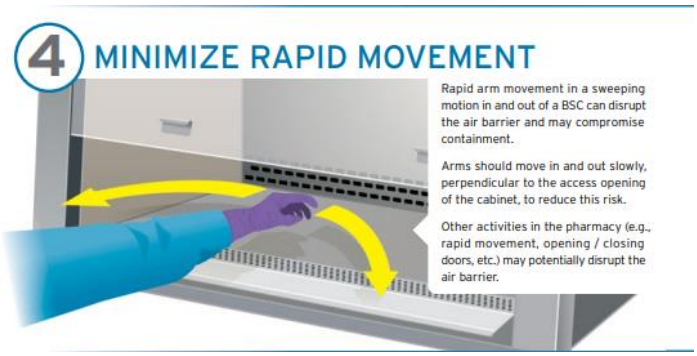


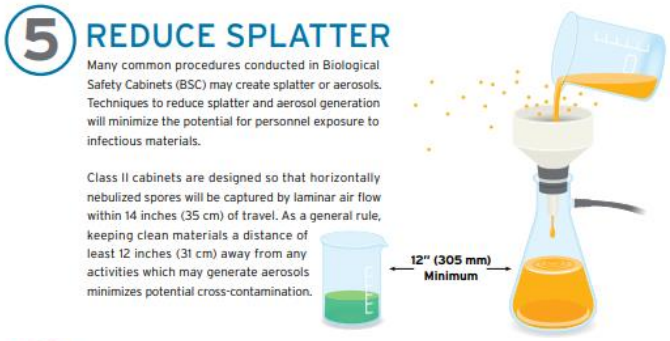


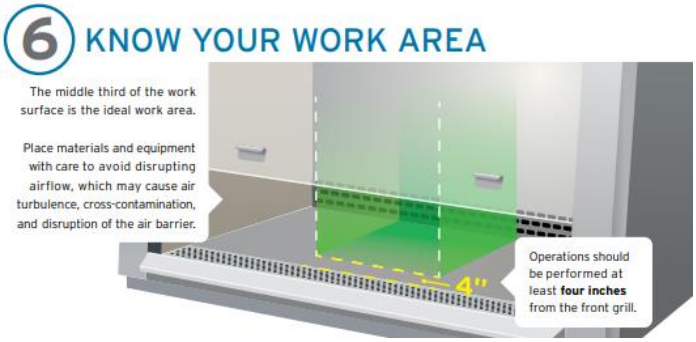


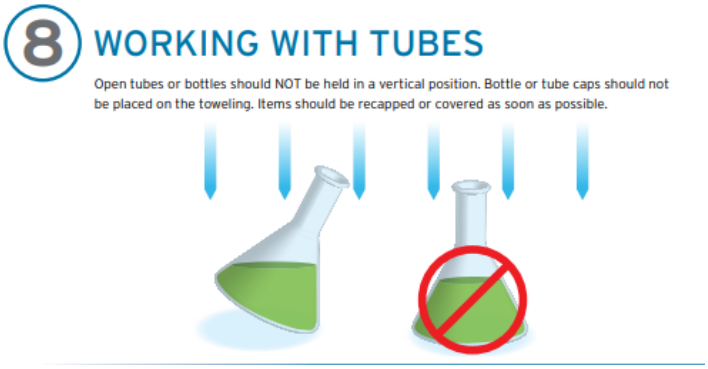
During Use:

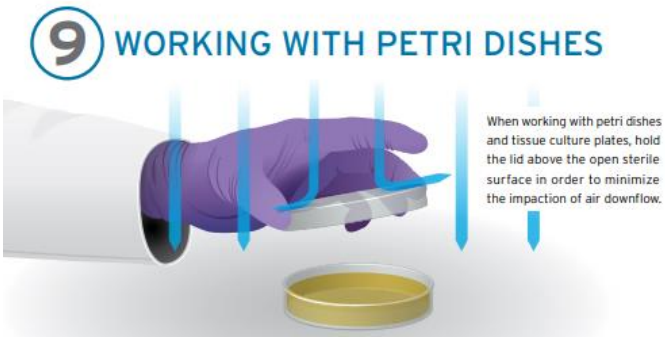
- Keep materials at least 4 inches inside work area.
- Minimize rapid movement as it disrupts the air barrier.
- Reduce splatter and potential aerosol creation.
- Work should proceed from clean to contaminated areas.
- Take precautions to prevent contamination of samples.
- Ensure vacuum lines and/or pumps are protected with an in-line HEPA filter.











After Use:

- After procedure completion, 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 and have the UV wavelength verified during certification if it will be used for germicidal disinfection. UV lamps may damage eyes, skin, and laboratory equipment. As such, many BSC manufacturers employ an interlock with the UV light and protective sash so that the user is not exposed to harmful light. 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 and difficulty in verifying germicidal effectiveness.

UV lamps may not be used as the primary means of decontamination. UV lamps may be used as a supplement after an appropriate chemical disinfectant has been used.

10.2 Procedures for Centrifugation

All centrifugation with biohazardous materials 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 BSC. Consult with the Biosafety Office prior to placing a centrifuge inside a BSC.

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

For high-speed machines, keep a logbook 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 BSC.
- 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 before each use. 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 BSC. If the rotor does not fit in the BSC, 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.

10.3 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 assembly 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 by pouring down sink and flushing with water. Alternative vacuum collection systems are provided on the EHS website.
- Vacuum line filters shall be examined before each use 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 11) shows an example of the proper assembly of a vacuum aspiration system, including placement of traps and a filter, is copied from the current version of the BMBL.

Figure 11. Protection of a house vacuum

Example method to protect a house vacuum system during aspiration of infectious fluids. The 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.

10.4 Syringes and Needles

Needles and syringes are the most common causes of injuries and exposure incidents. To lessen the chance of accidental injection, aerosol generation, or injuries to waste handlers, the use of syringes should be avoided when alternate methods are available. For example, use a blunt needle, pipette tip, or cannula on the syringe for blood collection, inoculation, or fluid administration and never use a syringe and needle as a substitute for a pipette in making dilutions.

The following practices are recommended for the use of hypodermic needles and syringes in research laboratories when performing biohazardous experiments:

- Use the syringe and needle in a BSC 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. Replace glass syringes with plastic disposable syringes whenever possible.

- Use syringes with safety devices, such as needle-locking syringes, whenever possible and be sure that the needle is securely shielded prior to discarding.
- Wear exam style 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 into a small tube containing sterile cotton with the appropriate disinfectant.
- 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.
- Connecticut state law requires syringes to be discarded into a needle box. DO NOT bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable and approved, use a one-handed method. DO NOT discard syringes into the EHS large biomedical waste container, biohazard bag, or box-bag unit.

10.5 Pipettes

The following is excerpted from *Laboratory Safety, Principles and Practices*, 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 (BSC).
- Mouth pipetting should be prohibited even with mouth pipetting devices that use a 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 of the BSC. Removing contaminated pipettes from the BSC 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 filled with an appropriate disinfectant for used pipettes within the BSC.
- 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.

10.6 Blenders, Mixers, Sonicators, and Cell Disruption Equipment

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

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 BSC.
- 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 infected tissues or materials with any open device is best done within a BSC.

10.7 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 materials 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 BSC. 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 (PI) should work out the protocol for each machine in consultation with the Biological Safety Officer (BSO). All people using the procedure must then follow the protocol.

10.8 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 (BBP) training. Fixatives take time to penetrate tissue; the fixatives may not inactivate pathogens deep in the tissue. Freezing and drying do not inactivate most pathogens, so, as with fixative use, the pathogens that may be present in the tissue should be considered capable of causing infection.

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

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

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

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

Things to remember when using and maintaining microtomes/cryostats:

- Always keep hands away from blades.
- Use extreme caution when aligning blocks, the blocks may be close to the blades. If available, make sure block holder is in locked position when loading/aligning blocks.
- Use knife-edge protectors/guards. Do not leave knife-edges that may extend beyond microtome knife holder unprotected.
- Keep blocks wet when in the microtome to minimize airborne shavings during slicing.
- Use brushes to clean/brush equipment.
- Use engineering controls such as forceps when removing or changing the blade.

- Dislodge stuck blocks using mechanical means such as forceps and/or dissecting probes.
- Wear appropriate PPE such as a lab coat or gown, mask, safety glasses or goggles, surgical grade Kevlar gloves that provide dexterity and cut protection, and examination gloves to protect against biohazards.
- When changing blades, wear ANSI-rated cut resistant 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.

10.9 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.as 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 appropriate 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 absorbent material.

No person should work alone on an extremely hazardous operation.

11 Decontamination and Disposal Procedures

11.1 Decontamination Methods

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

11.1.1 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 waste.

The hazards of handling hot solids and liquids are well recognized. 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.

11.1.2 Liquid Decontaminants

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

The use of chemicals for inactivation of biohazards requires successful completion of 5 different elements. These 5 factors, which all begin with the letter C, are:

1. Cleaning
2. Chemical
3. Concentration
4. Contact time
5. Coverage

These 5 Cs of disinfection are important to consider ensuring successful decontamination. Surfaces that are to be disinfected must be cleaned, as organic and other debris can consume the active ingredient in disinfectants or otherwise interfere with the chemical's effectiveness. The chemicals selected must be documented to be effective against the biohazard to be treated. It is also important to know what concentration of the chemical is effective and there are significant differences among the disinfectants. Chlorine based disinfectants do not lose effectiveness with increased concentrations, but iodine and alcohols will. Alcohols must be diluted with water for protein denaturation. Undiluted concentrations are not as effective or have much longer contact times. Iodine must be diluted to cleave the iodine which is bound to itself. Bound iodine has not effectiveness, until added to water to break the bonds to release free iodine for disinfection. Undiluted iodine has zero effectiveness.

Disinfectants do not work instantaneously. Contact time is needed and the length of time for successful inactivation will vary with different types of biohazards and conditions. Coverage refers to ensuring that all surfaces are adequately coated or in contact with the disinfectant.

Biohazards that are the most difficult to inactivate include prions or transmissible spongiform encephalopathies, and small molecular weight toxins. Parasitic cysts, spores and non-enveloped viruses

represent a category of very difficult to inactivate biohazards. Easier to inactivate pathogens include non-enveloped viruses and vegetative bacteria.

Information on disinfectants can be obtained from guidance documents from the NIH Office of Science Policy (NIH OSP). Their Laboratory Biosafety Manual contains a chart on effectiveness of various disinfectants against families of microorganisms. Their information has been summarized into the chart at the end of this section. Health Canada's Pathogen Safety Data Sheets (PSDS) also provide effective disinfectant information for over 200 pathogens in their database. Canadian PSDS can be accessed at this site.

The United States Environmental Protection Agency (EPA) has a series of lists of disinfectants effective against various biohazards. This list can be accessed at the EPA website.

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 (IA).

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 (PPE) to use as well as the toxicity associated with ocular, skin and respiratory exposure.

11.1.3 Vapors and Gases

A variety of vapors and gases possess decontamination properties. The most useful of these are vaporized hydrogen peroxide and chlorine dioxide. 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 (BSC) 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 hydrogen peroxide and chlorine dioxide. 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. Hydrogen peroxide and chlorine dioxide are acutely toxic at the concentrations used for vaporous/gaseous decontaminations. An outside contractor performs these decontaminations to minimize potential exposure to Yale University employees.

Use of vaporized hydrogen peroxide and chlorine dioxide is monitored closely by EHS. Please contact Yale EHS at 203-785-3550 for more information.

11.2 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 and moisture stable, provided that the steam can adequately penetrate the load. 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 203-785-3550.

Personal protection equipment (PPE) such as lab coats, rubberized aprons, impact resistant 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 EHS autoclave safety poster for additional information.

11.3 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.
- Lysis.
- 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 ingredients, 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.

11.3.1 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 exhibit antimicrobial action by denaturing proteins and are somewhat slow in germicidal action. However, alcohols are effective decontaminants against lipid-containing viruses. A contact time of 10 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. Alcohols are not effective against bacterial spores. Large amounts of ethanol should not be used in a BSC due to accumulation of vapors potentially leading to explosions.

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 solutions is an effective space decontaminant for buildings or rooms, but 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 humidity, gaseous formaldehyde produces fewer polymerized residues on surfaces, and treated areas can be cleared of residual fumes more quickly than when formaldehyde is used in its vaporized state.

Phenolics

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. Phenolic disinfectants are generally effective in the presence of organic material, but can be corrosive, toxic, and irritating to the eyes, skin, and respiratory tract.

Quaternary Ammonium Compounds or Quats

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

Chlorine

This halogen is a broad-spectrum decontaminant that is 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 due to their rapid degradation when exposed to light, organic material, and impurities commonly found in tap water. Chlorine leaves behind a non-toxic residue, making it suitable for many laboratory and facility decontamination purposes. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An effective decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25%, (52,500 ppm) available chlorine. When diluted 1:100, the resulting solution will contain 525 ppm of available chlorine. Adding a nonionic detergent at a concentration of about 0.7% enhances surface wetting and creates a highly effective decontamination solution.

Iodine

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

11.3.2 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 additional challenges, as agar, proteinaceous nutrients, and cellular materials can interfere with 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 menstrua. 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.

11.3.3 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. Compounds	0.1-2.0%	+	+			+					+	+	+	+		+	+	+	+	+	
Phenolic Compounds	1.0-5.0%	+	+	b		+	+			+			+	+		+	+	+	+	+	
Chlorine Compounds	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 10 minutes is generally used in efficacy testing of disinfectants.

12 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.

12.1 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 (BSL-2) or higher and for groups working with large volumes (≥ 1 liter) of BSL-1 material. A basic spill kit should include:

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

Additional personal protective equipment (PPE), such as Tyvek jump suits and powered air-purifying respirators (PAPRs), may be required for response to spills in BSL-3 laboratories.

Representatives from Environmental Health and Safety (EHS) are available if you have any questions regarding biological spill response procedures or decontamination by calling 203-785-3550. All spills in a BSL-3 laboratory shall be reported to EHS immediately.

12.2 BSL-1 Spill

- Notify others in the area, to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing and wash exposed skin with soap and water for 15 minutes.

12.2.1 Clean-up of BSL-1 Spill

- Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill allowing it to mix with spilled material. Allow 10-minute contact time.
- Pick up any pieces of broken glass with forceps and place in a sharps container.
- Discard all disposable materials used to clean up the spill into a biohazard autoclave bag.
- Wash hands with soap and water for 30 seconds.

12.3 BSL-2 Spill

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with a warning sign.
- Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag.
- Wash all exposed skin with soap and water.
- Inform Supervisor, and, if assistance is needed, consult the Biosafety Office at 203-785-3555.

12.3.1 Reporting of Spills Involving rDNA Materials or Human Pathogens

Immediately report to EHS any spills, exposures, or accidents involving spills of moderate to high risk RG2 or RG3 material, such as human, animal, or plant pathogens, recombinant DNA materials, biological toxins,

and any agents requiring registration with the CT DPH for assistance with the spill clean-up. EHS will assist in reporting the incident to the NIH Office of Science Policy (NIH OSP) as required under the NIH Guidelines, and other regulatory agencies as applicable.

12.3.2 Clean-up of BSL-2 Spill

- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, paper towels, biohazard bags, and forceps).
- Put on protective clothing (lab coat or Tyvek, face protection, utility gloves, and booties if necessary). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask.
- Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least a 10-minute contact time.
- Pick up any sharp objects with forceps or tongs and discard in a sharps container.
- Wipe surrounding areas (where the spill may have splashed) with disinfectant.
- Soak up the disinfectant and spill and place the materials into a biohazard bag.
- Spray the area with 10% household bleach solution and allow to air-dry (or wipe down with disinfectant-soaked towels after a 10-minute contact time). Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.
- Wash hands and exposed skin areas with disinfectant or antiseptic soap and water.

12.3.3 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 (or Tyvek lab coat).
- Absorb blood with paper towels or disinfectant-soaked 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 10 minutes.
- After the 10-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. Decontaminate any reusable items with disinfectant.
- Wash your hands with soap and water.

12.4 BSL-3 Spill

Please note that researchers should **not** attempt to clean up spills in BSL-3 labs. Individuals are expected to exit the BSL-3 laboratory within 1 minute, notify others to evacuate, remove PPE in the anteroom, notify EHS via the Emergency line at 203-785-3555, and post a sign indicating that there is a spill in the room and not to enter. See the BSL-3 Biosafety Manual for additional information.

12.5 Spill of a Biohazardous Radioactive Material

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

Before any clean up, consider the type of radionuclide, characteristics of the microorganism, and the volume of the spill. Contact the EHS Radiation Safety Office at 203-785-3555 for isotope clean-up procedures.

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door and post a warning sign.
- Remove contaminated clothing, turn exposed areas inward, and place in a biohazard bag labeled with a radioactive materials label or a radioactive waste container labeled with a biohazard label.
- Wash all exposed skin with disinfectant, following it with a three-minute water rinse.
- Inform supervisor and Radiation Safety Office of spill and monitor all exposed personnel for radiation. If assistance is needed in handling the microorganism, contact the Biosafety Office at 203-785-3555.

12.5.1 Clean-Up of a Biohazardous Radioactive Material

Do not attempt to clean up mixed hazard spills on your own. Immediately contact the EHS emergency line at 203-785-3555 and ask for a radiation safety subject matter expert.

- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, autoclavable containers, forceps, towel, and sponges) and confirm with the Radiation Safety Office at 203-785-3555 that it is safe to enter the lab.
- Put on protective clothing (gown, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA-filtered respirator instead of a surgical mask.
- Cover the area with disinfectant-soaked towels and carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least 10 minutes contact time.
- Do not use bleach solutions on iodinated material, radioactive gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.
- Handle any sharp objects with forceps. Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- Soak up the disinfectant and spill, and place the biologically decontaminated waste, along with all contaminated protective clothing, into an approved radiation container and label it according to Radiation Safety Guidelines. Contaminated protective clothing must also be biologically decontaminated prior to disposal as radioactive waste.

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

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

13 Select Agents

Select agents are materials that have been identified by the U.S. Government as biological agents and toxins that have the potential to pose a severe threat to public health and safety, animal health and safety, plant health and safety, or to the safety of animal or plant products. The Department of Health and Human Services (HHS), 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 under the following Code of Federal Regulations:

7 C.F.R. Part 331: Agriculture
9 C.F.R. Part 121: Animals and Animal Products
42 C.F.R. Part 73: Public Health

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. These agencies are required by law to review and republish the list of Select Agents and toxins on at least a biennial basis. The current list of select agents is below and can also be accessed from the Federal Select Agent Program (FSAP) website.

During the biennial review, input is received from previous Notices of Proposed Rulemaking, subject matter experts on federal advisory boards, and public comments. Final Rules from HHS and USDA are published in the *Federal Register*. The latest changes have taken effect as of January 16, 2025. These include:

- **Five select agents, including three of the *Brucella* species, are being removed from the list of regulated select agents and toxins:**
    - Includes 3 overlap select agents – *Brucella abortus, Brucella melitensis*, and *Brucella suis.*
    - Includes 2 USDA-only select agents – African horse sickness virus and *Peronosclerospora philippinensis* (also known as *Peronosclerospora sacchari*).
- **Implements several other changes to select agents and toxins already on the list:**
    - Updates nomenclature for Ebola virus to the genus *Ebolavirus,* and SARS coronavirus (SARS-CoV) to Severe acute respiratory syndrome coronavirus.
    - Designates Nipah virus as a Tier 1 select agent.
    - Increases the exclusion amount for Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$) from 100 mg to 200 mg.

For more information on the most recent update and past updates, please visit Changes to The Select Agent and Toxin List.

Yale University's current select agent program is in conformity with all conditions required under the current regulations. Entities and researchers in possession of these materials have additional obligations and responsibilities for their safe storage, use, transfer, and disposal.

13.1 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 Yale Environmental Health & Safety (EHS).

- Register with the Connecticut Department of Public Health (CT DPH) 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 (FBI), 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.

13.2 List of Select Agents and Regulated Toxins

The current list of Select Agents below is current as of June 2026. The Select Agent List is available online as well as the Select Agent Regulations, and the list of excluded agents and toxins.

HHS Select Agents and Toxins

1. Abrin [6]
2. *Bacillus cereus* Biovar *anthracis* [1]
3. Botulinum neurotoxins [1][6]
4. Botulinum neurotoxin producing species of *Clostridium* [1]
5. Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$) [6]
6. *Coxiella burnetii* [8]
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol [6]
9. Eastern equine encephalitis virus [4][5]
10. *Ebolavirus* [1]
11. *Francisella tularensis* [1]
12. Lassa fever virus
13. Lujo virus
14. Marburg virus [1]
15. Monkeypox virus [4]
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 [6]
18. *Rickettsia prowazekii*
19. Severe acute respiratory syndrome coronavirus (SARS-CoV) [5]
20. SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors
21. Saxitoxin [6]

South American Haemorrhagic Fever viruses:

22. Chapare
23. Guanarito
24. Junín
25. Machupo
26. Sabia
27. Staphylococcal enterotoxins (subtypes A,B,C,D,E) [6]
28. T-2 toxin [6]
29. Tetrodotoxin [6]

Tick-borne encephalitis complex (flavi) viruses:

30. Far Eastern subtype [5]
31. Siberian subtype [5]
32. Kyasanur Forest disease virus [5]
33. Omsk hemorrhagic fever virus [5]
34. Variola major virus (Smallpox virus) [1]
35. Variola minor virus (Alastrim) [1]
36. *Yersinia pestis* [1]

Overlap Select Agents and Toxins

37. *Bacillus anthracis* [1]
38. *Bacillus anthracis* Pasteur strain
39. *Burkholderia mallei* [1]
40. *Burkholderia pseudomallei* [1]
41. Hendra virus
42. Nipah virus [1]
43. Rift Valley fever virus
44. Venezuelan equine encephalitis virus [4][5]

USDA Veterinary Services (VS) Select Agents and Toxins

45. African swine fever virus
46. Avian influenza virus [4]
47. Classical swine fever virus [5]
48. Foot-and-mouth disease virus [1][5]
49. Goat pox virus
50. Lumpy skin disease virus
51. *Mycoplasma capricolum* [4]
52. *Mycoplasma mycoides* [4]
53. Newcastle disease virus [3][4]
54. Peste des petits ruminants virus
55. Rinderpest virus [1]
56. Sheep pox virus
57. Swine vesicular disease virus [5]

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

58. *Coniothyrium glycines* (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)
59. *Ralstonia solanacearum* [7]
60. *Rathayibacter toxicus*
61. *Sclerophthora rayssiae* [7]
62. *Synchytrium endobioticum*
63. *Xanthomonas oryzae*

13.3 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**
1. *Bacillus cereus* Biovar *anthracis* [1] 2. Botulinum neurotoxins [1][6] 3. Botulinum neurotoxin producing species of *Clostridium* [1] 4. *Ebolavirus* [1] 5. *Francisella tularensis* 6. Marburg virus [1] 7. Variola major virus (Smallpox virus) [1] 8. Variola minor virus (Alastrim) [1] 9. *Yersinia pestis* [1]	1. *Bacillus anthracis* [1] 2. *Burkholderia mallei* [1] 3. *Burkholderia pseudomallei* [1] 4. Nipah virus [1]	1. Foot-and-mouth disease virus [1][5] 2. Rinderpest virus [1]

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 security 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 Tier 1 Select Agents.

13.4 Permissible Toxin Amounts

The following toxins are not regulated if the amount under the control of a Principal Investigator (PI), 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	200 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. Yale's internal watch list limits PIs to only **50%** of the quantities shown here.

13.4.1 Toxin due Diligence Requirements

All Yale PIs 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).

13.4.2 Reporting Suspected Violations or Suspicious Activity

If a Yale PI in possession of **any quantity** of the Select Agents or Toxins listed above detects suspicious activity associated with a request for agent or toxin or suspicious activity associated with a shipped agent or toxin, they must immediately notify the Yale University Select Agent Program Responsible Official (RO) or Alternate Responsible Officials (ARO).

Role	Name	Office	Cell
Responsible Official	Benjamin Fontes	203-737-5009	203-410-6223
Alternate Responsible Official	Maren Schniederberend	203-737-7959	203-507-0028
Alternate Responsible Official	Melissa James	203-737-4228	412-551-9068
Alternate Responsible Official	Anthony D'Abramo	203-737-2114	860-384-3864
Alternate Responsible Official	Julia Kontos	N/A	475-331-0650
Alternate Responsible Official	Emily Bludnicki	N/A	475-224-1550
Alternate Responsible Official	Kevin Charbonneau	203-737-2139	203-410-8527
Alternate Responsible Official	John Sterpka	N/A	203-228-1603

To report safety, security, or other concerns associated with select agents and toxins, please use the Office of Inspector General (OIG)'s confidential hotline. When reporting these issues to OIG, please ensure that you indicate it is a "Select Agent Complaint" issue.

HHS OIG Hotline contact information

Voice: 1-800-447-8477
Fax: 1-800-223-8164
HHS OIG Website

Mail:
Office of Inspector General
Department of Health & Human Services
Attn: Hotline
P.O. Box 23489
Washington, DC 20026

USDA OIG Hotline contact information

Voice: 1-800-424-9121
Fax: 1-202-690-2474
USDA OIG Website

Mail:
United States Department of Agriculture
Office of Inspector General
P.O. Box 23399
Washington, DC 20026-3399

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

All activity involving Select Agents must be registered with the Yale Environmental Health & Safety prior to initiation. Please contact the Biosafety Office with Yale EHS or contact your EHS Safety Advisor (SA) at 203-785-3550 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 CT DPH).
- 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 (BBP), Laboratory Safety, Biosafety Level 3 (BSL-3), Select Agent Biosecurity, and Shipping/Transport of Hazardous Biological Agents).
- Complete a Yale EHS request to use Infectious Agents (IA) form(s) and Researcher Experience Form.
- Complete the Yale EHS researcher experience requirements (for BSL-2+ or BSL-3).
- 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, Yale IBC, CT DPH, and FSAP for addition of personnel, the proposed storage location for Select Agents, and the Select Agent research procedures. **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.

13.6 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.

13.7 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 at 203-785-3550 for additional information.

13.8 Destruction of Select Agents or Unknown Samples

Select Agent pathogens or toxins discovered in unregistered labs 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 Federal Select Agent process, please don't hesitate to contact the EHS Biosafety Office or your EHS SA at 203-785-3550.

13.9 Additional Information

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

Yale EHS

FSAP

Select Agents and Toxins Exclusions

14 Research Involving Recombinant and Synthetic Nucleic Acid (r/sNA) Molecules

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids* (r/sNA) (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.

14.1 Principal Investigator Responsibilities Under the NIH Guidelines

Principal Investigators (PI) 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 as part of their written risk assessment.
- Obtaining authorization from the Yale Biological Safety Committee prior to starting any research that is subject to the NIH Guidelines.

The NIH has created a brochure for Principal Investigators which outlines their responsibilities as noted within the NIH Guidelines. The brochure entitled, "Investigator Responsibilities Under the NIH Guidelines for Research Involving Recombinant DNA Molecules," can be accessed at this web link.

Research with recombinant or synthetic nucleic acid molecules (r/sNA) is split into non-exempt and exempt categories. However, as the Yale IBC reviews a wide range of biohazardous and biological materials research, both exempt and non-exempt r/sNA research must be registered and authorized by the IBC before research may begin.

The definition of r/sNA Molecules directly from the NIH Guidelines, Section I-B, defines recombinant and synthetic nucleic acids 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 acid), or

(iii) molecules that result from the replication of those described in (i) or (ii) above.

Non-exempt recombinant DNA experiments must be registered and approved prior to initiation. Higher risk rDNA experiments that require registration with the Yale Biological Committee (IBC) and at least one other regulatory committee include the following:

- Deliberate transfer a drug resistance trait to a microorganism.
- Human gene transfer (HGT).
- Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of <100 µg/kg body weight.

Other rDNA experiments that require registration and approval from the IBC include:

- Human or animal pathogens as host-vector systems.
- Cloning of DNA or RNA from certain pathogens.

- Recombinant DNA work in whole animals or plants.
- Gene drive experiments.
- 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 at the Yale EHS website. To access the complete set of the current NIH Guidelines, please visit this webpage.

Additional information on the NIH Guidelines can be found in Chapters 17 and 18 of this manual.

Yale EHS has created a series of help documents describing experiments with recombinant or synthetic nucleic acid molecules for PIs, lab managers, and researchers. These help documents are described below with the link to access them for additional information.

- The first help document is entitled, "Research Involving Recombinant or Synthetic Nucleic Acid (r/sNA) Molecules," and includes the definition of r/sNA experiments and provides many examples of the types of experiments that require registration and lists their classification from the NIH Guidelines.
- The "Protocol Tracing for Institutional Biosafety Committee (IBC) Protocols" document provides labs with an overview of the steps involved in the submission, review and approval of r/sNA protocols by the IBC. All of the steps involved are related to an aspect of a regulatory, policy or committee requirement. This form is helpful in assisting labs with awareness of all of the documentation and steps involved in the protocol review process.
- Another help document, entitled, "Overview of Recombinant DNA Experiments Covered by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) April 2024," provides in depth descriptions of the various classifications of research within the NIH Guidelines and also includes the details of prior updates related to synthetic nucleic acids and gene drive experimentation.
- Yale EHS has created a very brief 1-page help document titled, "Recombinant DNA Experiments Registration and Approval Guidelines," for a very quick overview of the experiments which require review and authorization prior to initiation of work.
- The NIH created very descriptive tables of r/sNA research involving animals entitled, "rDNA Animal Experiments Covered Under the NIH Guidelines," for your review and use.
- For those researchers who are conducting clinical trials involving r/sNA molecules, review the following two EHS documents that explain the entire human gene transfer registration process from protocol submission to approval and includes the registration form that must be submitted to initiate protocol review.
    - Human Gene Transfer Clinical Trials, Appendix F
    - Human Gene Transfer Registration
- EHS has created a very extensive set of examples of r/sNA experiments by the various classifications within the NIH Guidelines for your awareness and review. See the document entitled, "Table of Experiments Described in the NIH Guidelines by Section and Example," for more information.

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 misclassified by PIs.

Please also review the NIH Guidelines for more information or contact your EHS Safety Advisor and/or Biosafety Office representative at 203-785-3550 if you have any questions. You can also email questions related to the NIH Guidelines to the NIH Office of Science Policy: NIHGuidelines@od.nih.gov.

14.1.1 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, IRB, and FDA Approval	Introduction of rDNA into human subjects (Human gene transfer – HGT).	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

15 Shipping

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

- United States Department of Transportation (DOT).
- International Civil Aviation Organization (ICAO).
- 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 within 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.

15.1 Training

15.1.1 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 on the EHS website that outlines the training requirements based on the material being shipped.

15.1.2 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.

15.2 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 shipping@yale.edu or 203-785-3550.

Packaging for human or animal materials is available at the Medical School and Kline Stockrooms. Additional packaging options are also available through SciQuest.

15.3 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.

15.4 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 at 203-785-3550 if you need to transport materials between Yale campuses.

15.5 Exports and Imports

15.5.1 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, EHS staff will apply for the license on behalf of the PI. See the EHS website or additional information and resources on exporting research materials.

15.5.2 Imports

The CDC, the USDA, and other agencies have import permit requirements. See the EHS website for additional information on importing research materials. For assistance, please contact the Biosafety Office at 203-785-3550 or the appropriate agency.

15.5.3 Table of Required Permits

Agency	Material to be Imported
CDC Call 404-639-3883 or visit the CDC website at CDC Import Permit Program for further information	**Etiologic agents -** Infectious agent (IA) 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 (NHP) 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 website at USDA Permit 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 NHP 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. • Soil and materials containing soil may be regulated on a county-by-county basis. • 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 website at FWS Permits for further information	Certain live animals and all live bats

16 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 counterproductive. 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 Affairs Section (EAS) is continually working behind the scenes to improve this program and to control its cost. Direct any questions or suggestions to the Environmental Affairs Waste Line at 203-432-6545 or send an email to waste.requests@yale.edu. 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 graduated 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 provided sharps container/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 brown 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. No liquids, chemicals, radioactive materials, broken glass, aerosol or large metal pieces should be placed in the box-bag unit.
- When the box-bag unit is full, seal the red bag, attach an address label (available at the stockrooms), seal the box according to the instruction provided in the trainings and printed on the box itself. 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 the box-bag units inside the laboratory. Compliance with fire codes and maintaining control of this special waste stream is very important.

Medical School

Medical waste box/bag units can also be brought to a designated EAS collection location (review EHS website for more information), or a request can be submitted via EHS Integrator under bio waste request.

Science Hill, Central Campus, and West Campus

- Medical waste box/bag unit removal requests must be submitted via EHS Integrator.
- Sharps containers, autoclave bags and box-bag units shall only be used for medical waste disposal.

Ordering Procedures

- Extra box-bag units and red sharps containers can be obtained through a submitted EHS Integrator request and delivered to your lab by EAS at no cost to your laboratory.
- Benchtop sharps containers are available at either the Medical School, BASS or West Campus stockrooms at no cost to your laboratory.
- Autoclave bags may be purchased at either the Medical School, BASS or West Campus stockrooms.

D. Definition of Medical Waste

Medical waste is defined using the following criteria:

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

- Cultures and stocks of infectious agents (IA) or microorganisms from facilities assigned to Biosafety Levels 1 through 3 (BSL-1, BSL-2, BSL-3).
- Cultures of specimens from medical and pathological laboratories.
- Disposable containers, materials, and supplies that may have been contaminated during the manipulation of microbial cultures and stocks.
- Wastes from the production of biologicals (including all tissue culture materials).
- Live and attenuated vaccines.

2. Human Pathological Wastes

Pathological waste consists of human and animal 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

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

4. Used Sharps Waste

This category includes any items that can puncture a cardboard box, including 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 Risk Group 1, 2 or 3 agents shall be autoclaved. Use the pre-existing disposal system implemented by the Yale Animal Resource Center (YARC).

Uninfected or "clean" animals shall be wrapped in a nondescript plastic bag and discarded into grey barrels in YARC 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 RG4 agents.

If a human or animal is known to be infected with a RG4 agent, contact the Biosafety Office at 203-735-3555 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 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 (RG 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. Sharps Containers

- Discard all intravascular sharps waste such as hypodermic needles, syringes (with/without the attached needles), scalpel blades, and suture needles in your sharps 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 Containers

Do not discard needles, syringes or other intravascular sharps into a red container. 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 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. Sharps containers should 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 (PSI) pressure for 60 minutes. Drain excess liquid and allow it 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 sharps container, red bio container and label accordingly. When the container is full, place it directly into a Stericycle box-bag unit. Do not autoclave or bleach decontaminate.

2. Chemotherapy Waste and Medical Waste

Labs should contact the EAS waste line for guidance on disposal of Chemotherapy drug since some are considered hazardous even if only trace amounts remain.

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

Call EAS at 203-432-6545 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 EAS. These containers can be requested via the Radiation waste page in EHS Integrator.

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

4. Other Mixed Waste Issues

If you have any questions regarding other mixed waste issues, please call EAS at 203-432-6545.

I. Sharps Containers

General Procedures

- All non-radioactive hypodermic needles, syringes (with or without the attached needles), and other intravascular sharps waste must be discarded into sharps containers.
- Other non-radioactive sharps waste that was in contact with IAs or other biologicals may be deposited into sharps containers.
- Containers may be used freestanding or wall mounted. Order containers and wall brackets through the EHS Integrator.
- 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, DAB, phorbol or phenol-chloroform mixtures are already chemically decontaminated. Segregate these sharps into one container and label accordingly. Do not autoclave or bleach.

- **Bleach Decontamination**: Fill the container with a 1:10 dilution of bleach. Push the flap on top of the container into the opening and past the first or second set of stops. Allow the bleach solution to stand in the container overnight. Invert the container in a sink to drain off the bleach solution.
- **Autoclaving is preferred when feasible**: 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 PSI of pressure for 60 minutes.

Disposal

After biological decontamination, place container upright into Medical Waste box-bag unit. There are different procedures depending on which campus you are on:

- **Medical School:**
  - Medical waste box/bag units, non-path sharps and bio containers should be brought to an EAS collection location (review EHS website for more information).
  - Stericycle boxes used for pathological waste can also be brought to a designated EAS collection location, or a request can be submitted via EHS Integrator under bio waste request.
- **Science Hill, Central Campus, and West Campus:**
  - BSL-1 waste should be autoclaved and treated as regular trash.
  - For all material classified at BSL-2 or higher, the laboratory must submit a bio waste request via EHS Integrator.

J. Red Sharps Containers

General Procedures

Non-radioactive, non-intravascular sharps shall be deposited into a red bio 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 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 EHS Integrator. Benchtop needle boxes are available in the campus stockrooms free of charge.

Biological Decontamination

All sharps containers shall be biologically decontaminated.

- **Chemical Decontamination**: Sharps contaminated with ethidium bromide, 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 whenever feasible**: 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 EAS at 203-432-6545 for a waste inspection.

17 April 2024 Changes to the NIH Guidelines: Gene Drive Modified Organisms

NIH has amended the NIH Guidelines to ensure the continued responsible research involving Gene Drive Modified Organisms (GDMOs) in contained research settings. The changes take effect at the end of September 2024.

Specifically, the NIH Guidelines will be amended to:

- Clarify minimum containment requirements for research involving Gene Drive Modified Organisms (GDMOs).
- Provide considerations for risk assessment.
- Define additional institutional responsibilities for IBCs and BSOs.

In addition to the amendments related to contained research involving GDMOs, the NIH Guidelines will also be amended to:

- Replace the term "helper viruses" with the broader term "helper systems."

The potential for reversion or generation of replication competent virus should be considered when generating or using defective viruses or vectors in the presence of helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems).

And

- Reclassify West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) as risk group (RG) 2 agents for consistency with containment guidance provided in the BMBL.

Gene Drive Modified Organisms (GDMOs)

Definition of a Gene Drive:
Section I-E-7. "Gene drive" is defined as a technology whereby a particular heritable element biases inheritance in its favor, resulting in the heritable element becoming more prevalent than predicted by Mendelian laws of inheritance in a population over successive generations.

Field release or field work with GDMOs not allowed:
NIH does not currently support field release of GDMOs and the NIH Guidelines pertain to contained research. All research involving GDMOs must be conducted within a research, plant or animal research laboratory after authorization from the Yale IBC.

GDMO Research Must be Registered with and Approved by the Yale IBC.

GDMO research is <u>not exempted</u> from the NIH Guidelines. Research subject to the NIH Guidelines, including research with GDMOs, requires review and approval by an IBC that is registered with the NIH Office of Science Policy (NIH OSP) prior to initiation. The following categories are not exempt from the NIH Guidelines:

- Experiments described in Section III-B, which require NIH OSP and Institutional Biosafety Committee (IBC) approval before initiation.

- Experiments involving DNA from RGs 3, 4, or restricted organisms (see Appendix B of the NIH Guidelines, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior IBC review and approval.
- Large-scale experiments (e.g., more than 10 liters of culture).
- Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates).
- Experiments involving gene drive modified organisms (Section III-D-8).

This means that any GDMO research in *E. coli* K12, s*accharomyces cerevisiae*, *B. subtilis*, purchased transgenic rodents, or other exempt research must be registered and approved by the Yale IBC prior to initiation.

GDMO Research Requires a Minimum of BSL-2 Containment:
Research involving GDMOs must be conducted at a minimum containment level of BSL-2, BL2-N (Animal BSL-2), or BL2-P (Plant BSL-2). Based on the risk assessment of a specific research protocol, the IBC may require enhancements or a higher level of containment. Review and approval from NIH OSP are required to lower containment below the minimum specified in the NIH Guidelines.

> **Section III-D-8.** Experiments Involving Gene Drive Modified Organisms
> Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid (r/sNA) molecules shall be conducted at a minimum of Biosafety Level (BSL) 2, BL2-N (Animals) or BL2-P (plant) containment.

When the institution conducts research involving GDMOs, the institution must ensure that the IBC has adequate expertise (e.g., specific species containment, ecological or environmental risk assessment) using ad hoc consultants if necessary. The Yale IBC has added consultants to the committee to assist with the ecological risk assessment of GDMO in the event of an accidental release from the laboratory.

In addition, when such research is being conducted, a Biological Safety Officer (BSO) shall be appointed to the IBC. Yale University's Biosafety Officer is a representative of the IBC.

Research involving gene drive modified organisms may require risk assessments that incorporate a broader scope of considerations because of greater uncertainty of the technology and potential uncertainty of the impact of the newly modified organism. Specific attention must be paid to risks of an unintended release from the laboratory and the potential impact on humans, other populations of organisms, and the environment.

Considerations for conducting risk assessments for research involving gene drive modified organisms might include:

The specific types of manipulations based on:
- Function or intended function of the genetic/gene drive construct (i.e., a designed or engineered assembly of sequences).
- Source of the genetic material (e.g., sequences of transgenes) in the construct.
- The modifications to the construct.

- Whether it is possible to predict the consequences of a construct, including the recognition of an unintended gene drive (i.e., construct not specifically designed as a gene drive but nonetheless having properties of a gene drive) and the possible consequences of escape into the environment,
- The potential ability of the gene drive to spread or persist in local populations;

Options for approaches to risk mitigation for specific types of risks in experiments or when dealing with a high degree of uncertainty about risks.

Considerations for implementing more stringent containment measures until biosafety data are accrued to support lowering containment.

The conduct of risk assessments for research involving GDMOs presents challenges in addition to those associated with other genetically modified organisms (GMOs) or vectors because the preferentially inherited traits of GDMOs spread and persist in the environment, are intended to modify natural populations, and may have associated impacts on the environment and society.

The potentially broad and long-lasting impacts of the use of this technology on humans, other populations of organisms, and the environment are not seen with research involving clinical research participant cohorts or even with other GMOs not designed to survive outside of laboratory containment. As such, research involving GDMOs requires risk assessments that incorporate a broader scope of issues because of the greater uncertainty in terms of risks in the event of an unintended release from the laboratory.

18 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 (HGT) is 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:
  - Contain 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 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).
- HGT 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:
    - 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
    - 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.

19 Abbreviations Used Throughout this Manual

Abbreviation	Stands for
ANSI	American National Standards Institute
APHIS	Animal Plant Health Inspection Service
BBP	Bloodborne Pathogen
BMBL	CDC/NIH Biosafety in Microbiological and Biomedical Laboratories
BSAT	Biological Select Agents and Toxins
BSC	Biosafety Cabinet
BSL	Biosafety Level
BSO	Biological Safety Officer
CAD	Clean Air Device
CDC	Centers for Disease Control and Prevention
DAB	Diaminobenzidine
DNA	Deoxyribonucleic acid
CT DPH	Connecticut Department of Public Health
DURC	Dual Use Research of Concern
EAS	Environmental Affairs Section
EHS	Environmental Health and Safety
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
FSAP	Federal Select Agent Program
HBV	Hepatitis B Virus
HEPA	High-Efficiency Particulate Air
HGT	Human Gene Transfer
HHS	Health and Human Services
HIV	Human Immunodeficiency Virus
IA	Infectious Agent
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
IRE	Institutional Review Entity
LD50	Lethal Dose 50%
LFCB	Laminar Flow Clean Bench
NHP	Non-Human Primate
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
OARS	Office of Animal Research Support
OIG	Office of the Inspector General
OPIM	Other Potentially Infectious Materials

OSHA	Occupational Safety and Health Administration
OSP	Office of Science Policy
PAPR	Powered Air-Purifying Respirator
PI	Principal Investigator
PPE	Personal Protective Equipment
PSDS	Pathogen Safety Data Sheet
PSI	Pounds per Square Inch
r/sNA	Recombinant or synthetic nucleic acid
RG	Risk Group
RNA	Ribonucleic Acid
SA	Safety Advisor
SDS	Safety Data Sheet
SOP	Standard Operating Procedure
TB	*Mycobacterium tuberculosis*
USDA	United States Department of Agriculture
UV	Ultraviolet
YARC	Yale Animal Resources Center
YNHH	Yale New Haven Hospital

20 Additional Helpful Resources

20.1 Biosafety Posters

Yale EHS Poster	Link
Autoclave Safety Poster	https://ehs.yale.edu/sites/default/files/files/autoclave-safety.pdf
BSL-1 Laboratory Practices Poster	https://ehs.yale.edu/sites/default/files/files/bsl1-lab-practices-english.pdf
BSL-2 Laboratory Practices Poster	https://ehs.yale.edu/sites/default/files/files/bsl2-lab-practices-english.pdf
BSL-2+ Work Practices QR Code Poster	https://ehs.yale.edu/resource/bsl-2-enhanced-work-practices-videos-qr-codes
Biological Toxin Poster	https://ehs.yale.edu/sites/default/files/files/hazardous-biological-toxins.pdf
Biosecurity Poster	https://ehs.yale.edu/sites/default/files/files/biosecurity.pdf
Breaks in Skin Poster	https://ehs.yale.edu/sites/default/files/files/skin-wounds.pdf
Biosafety Cabinet Checklist Poster	https://ehs.yale.edu/sites/default/files/files/bsc-checklist.pdf
BSC vs. Chemical Fume Hood Poster	https://ehs.yale.edu/sites/default/files/files/bsc-fume-hood.pdf
Centrifuge Safety Poster	https://ehs.yale.edu/sites/default/files/files/centrifuge-safety-english.pdf
Cryostat/Microtome Safety Poster	https://ehs.yale.edu/sites/default/files/files/cryostat-microtome.pdf
Incident Exposure Poster	https://ehs.yale.edu/sites/default/files/files/incident-exposure-card-english.pdf
Safety Manuals QR Code Poster	https://ehs.yale.edu/resource/safety-manuals-qr-codes
Spill Response Poster	https://ehs.yale.edu/sites/default/files/files/bbp-spill-response-english.pdf

20.2 Biosafety SOPs

Yale SOP	Link
BSL-2 Cell Culture SOP	https://ehs.yale.edu/sites/default/files/files/bsl2-cell-culture-sop.docx
BSL-2+ Work Practices SOP	https://ehs.yale.edu/sites/default/files/files/bsl2-plus-work-practices.docx
Biological Safety Policies and Procedures	https://ehs.yale.edu/biological-policies-procedures
YARC BSL-2 SOP	https://ehs.yale.edu/sites/default/files/files/yarc-bsl2-procedures.pdf
YARC BSL-2+ SOP	https://ehs.yale.edu/sites/default/files/files/yarc-bsl2-plus-procedures.pdf

Transgenic Zebrafish SOP	https://ehs.yale.edu/sites/default/files/files/transgenic-zebrafish-sop.pdf
Transgenic Drosophila SOP	https://ehs.yale.edu/sites/default/files/files/transgenic-drosophila-sop.pdf
Research Involving Prion-like Proteins	https://ehs.yale.edu/resource/research-involving-prion-like-proteins

20.3 Biosafety Ordering Information Guides

Yale EHS Ordering Help Guides	Link
Cell sorter purchasing information	https://ehs.yale.edu/sites/default/files/files/cell-sorting-purchase.pdf
Cell sorter containment testing	https://ehs.yale.edu/sites/default/files/files/vacuum-system-protection.pdf
Centrifuge rotor information	https://ehs.yale.edu/sites/default/files/files/centrifuge-rotors.pdf
Safety product order information	https://ehs.yale.edu/sites/default/files/files/ordering-information-safety-products.pdf
Safe sharp product ordering	https://ehs.yale.edu/sites/default/files/files/ordering-information-safe-sharps.pdf
Vacuum system set-up	https://ehs.yale.edu/sites/default/files/files/vacuum-system-protection.pdf

21 Index of Resources Referenced Throughout This Manual

Resource	Source	Link	Chapter
Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition.	CDC/NIH	https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf	6, 6.3
Canadian Biosafety Standard, Third Edition	Public Health Agency of Canada	https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/third-edition.html	6
2024 Updates to the Federal Select Agent Program	CDC FSAP	https://www.selectagents.gov/overview/whatsnew/2024.htm	13
Select Agents and Toxins Exclusions	CDC FSAP	https://www.selectagents.gov/sat/exclusions/index.htm	13.9, 13.2
Code of Federal Regulations for Select Agents – Agriculture	CFR	https://www.ecfr.gov/current/title-7/subtitle-B/chapter-III/part-331	13
Code of Federal Regulations for Select Agents – Animals and Animal Products	CFR	https://www.ecfr.gov/current/title-9/chapter-I/subchapter-E/part-121	13
Code of Federal Regulations for Select Agents – Public Health	CFR	https://www.ecfr.gov/current/title-42/chapter-I/subchapter-F/part-73	13
Biological Safety Tools and Resources	Yale EHS	https://ehs.yale.edu/biological-tools-resources	8.7.1, 8.81
Biosafety Notice Form	Yale EHS	https://ehs.yale.edu/sites/default/files/files/biosafety-equipment-notice-tag.pdf	7.4
BSL-3 Research/Select Agents & Dual Use Research of Concern	Yale EHS	https://ehs.yale.edu/select-agents-bsl-three	13.9
Researcher Competence Verification Form	Yale EHS	https://ehs.yale.edu/sites/default/files/files/researcher-competence-verification.pdf	6
Employee Insurance Claim Reporting	Yale	https://ogc.yale.edu/erm/reporting-insurance-claims?app=yalesites	5.2
Select Agents and Toxins List	CDC FSAP	https://www.selectagents.gov/sat/list.htm	13.9, 13.2, 13
Federal Select Agent Regulations	CDC FSAP	https://www.selectagents.gov/regulations/index.htm	13.2
High Risk Biomaterials Laboratory Exit Interview Checkout Sheet	Yale EHS	https://ehs.yale.edu/sites/default/files/files/hrb-lab-exit-interview.pdf	8.8.1
Laboratory Safety Monograph	NIH	https://www.cambridgepublichealth.org/wp-content/uploads/2022/09/Laboratory_Biosafety_NIH-Monograph-1979.pdf	8.8.3
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH GUIDELINES)	NIH OSP	https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf	15.1,14.1, 3.2, 3.3
NIH Guidelines Honoring the Past Charting the Future	NIH OSP	https://osp.od.nih.gov/biotechnology/nih-guidelines/	15.1
NIH Office of Science Policy	NIH OSP	https://www.osp.od.nih.gov	3.8.4

US Department of Health and Human Services Office of Inspector General Hotline	HHS	https://oig.hhs.gov/fraud/report-fraud/index.asp	13.4.2
US Department of Agriculture Office of Inspector General Hotline Portal	USDA	https://usdaoig.oversight.gov/hotline	13.4.2
OSHA Bloodborne Pathogens Standard	OSHA	https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030	8.8
Aerosols Generated During Lab Activities and Spills	ASM Journal	https://journals.asm.org/doi/10.1128/am.16.8.1146-1150.1968	8.7.1, 10.3
Pathogen Safety Data Sheets	Public Health Agency of Canada	https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html	6, 11.1.2
Safe Sharps Products Ordering Information Safety Guidelines	Yale EHS	https://ehs.yale.edu/sites/default/files/files/ordering-information-safe-sharps.pdf	10.4
Select Agent Toxin Inventory/Due Diligence Transfer Log	Yale EHS	https://ehs.yale.edu/resource/select-agent-toxin-inventory-due-diligence-transfer-log-for-yale-laboratories	3.8.2
Selected EPA-Registered Disinfectants	EPA	https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants	11.1.2
US Fish and Wildlife Service	USFWS	https://fwsepermits.servicenowservices.com/fws	16.5.3 Table
Workday Learning	Yale	https://www.myworkday.com/yale/learning	3.9
Yale 1430 PR.01 Shipping Policy Exception Requests	Yale	https://your.yale.edu/policies-procedures/1430-pr01-shipping-policy-exception-requests	8.6
Yale Animal Care and Use Committee (IACUC)	Yale	https://development-ys-research-support-yale-edu.pantheonsite.io/research-compliance/animal-research/submit-for-iacuc-review/managing-animal-protocols-and-studies?check_logged_in=1	3.6
Yale Biological Safety BSL-3 Lab Manual	Yale EHS	https://ehs.yale.edu/sites/default/files/files/bsl3-lab-manual.pdf	3.2
Yale Bloodborne Pathogens Training Manual	Yale EHS	https://ehs.yale.edu/sites/default/files/files/bbp-training-manual.pdf	3.5
Yale Clean Air Device Guide	Yale EHS	https://ehs.yale.edu/sites/default/files/files/clean-air-device.pdf	10
Yale EHS Integrator	Yale EHS	https://ehsis.yale.edu/EHSIntegrator/Registration	3.1, 3.2, 3.3
Yale Emergency Eyewash Instructions	Yale EHS	https://ehs.yale.edu/emergency-eyewash-videos	5.1.2

Yale Environmental Health and Safety Website	Yale EHS	http://www.yale.edu/ehs/	1, 2
Yale Exporting Research Materials	Yale EHS	http://ehs.yale.edu/exporting-research-materials	17.5.1, 16.5.1
Yale Guidelines for Human Gene Transfer Clinical Trials	Yale EHS	https://ehs.yale.edu/sites/default/files/files/human-gene-transfer-appendix-f.pdf	3.4
Yale HGT Registration Form	Yale EHS	https://ehs.yale.edu/sites/default/files/files/hgt-registration.pdf	
Yale Human Gene Transfer	Yale EHS	https://ehs.yale.edu/human-gene-transfer	3.4
Yale Importing Research Materials	Yale EHS	http://ehs.yale.edu/importing-research-materials	17.5.2, 16.5.2
Yale Overview of Recombinant DNA Experiments Covered by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2024.	Yale EHS	https://ehs.yale.edu/sites/default/files/files/rdna-experiments-nih.pdf	3.3
Yale Research Materials Shipping Training Requirements	Yale EHS	https://ehs.yale.edu/research-materials-shipping-training-requirements	17.1.1,16.1.1, 3.9 Table
Yale Respiratory Protection Program	Yale EHS	https://ehs.yale.edu/respiratory-protection	9.2
Yale Safety Product Ordering Information	Yale EHS	https://ehs.yale.edu/sites/default/files/files/ordering-information-safety-products.pdf	8.7.1
Yale Toxin Safety Poster	Yale EHS	https://ehs.yale.edu/sites/default/files/files/hazardous-biological-toxins.pdf	3.8.1
Yale University Infection Control Manual	Yale EHS	https://ehs.yale.edu/sites/default/files/files/infection-control-policy.pdf	4.1
Yale Workplace Health and Safety	Yale	https://your.yale.edu/working-at-yale/manager-toolkit/workplace-health-safety	5.2