Brandeis University Biosafety Handbook

IBC Revision 5.0 (FOS)
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I. INTRODUCTION

It is the policy of Brandeis University to maintain a safe and healthful workplace for all, and for those who work with recombinant/synthetic nucleic acids and infectious agents in particular. Biological research at Brandeis University must be carried out in accordance with the NIH Guidelines and all other applicable federal, state, and local regulations. The Brandeis University Biosafety Handbook is intended to be a concise guide to the regulations and recommendations by federal, state, and local governments for the user of recombinant/synthetic nucleic acids and biological materials. It is not intended to substitute for the complete *NIH Guidelines, April 2019*, *Biosafety in Microbiological and Biomedical Laboratories, 6th Edition*, or other biosafety regulations. Rather, it should be used to assist in navigating the contents of those documents.

This Handbook has also been developed to provide general policies and procedures when working with nucleic acids, tissues, cell lines, and microorganisms. Each individual laboratory, however, should supplement the information from this Handbook with laboratory-specific policies, procedures, and training that will minimize the risks unique to that laboratory.

Finally, the hazards present in any particular laboratory are not only limited to biological materials; chemical, radiological, and laser hazards are also of concern. Consequently, biosafety is to be viewed as one component of a total laboratory safety program. In line with this view, the Brandeis University Chemical Hygiene Plan (http://www.brandeis.edu/ehs/labs/) provides a general description of the physical and chemical hazards that may be encountered within a laboratory and the corresponding protective work practices, procedures, and controls. The particular laboratory may then tailor those safety procedures and equipment to meet their specific needs.

Additional information on safety issues may be obtained from the relevant Offices listed below.

- For general laboratory safety/chemical safety/shipping and packaging concerns, contact Mr. Andrew Finn, Director, the Department of Environmental Health and Safety (EH&S). afinn@brandeis.edu, or X6-4262
- For radiological and laser safety support, contact Mr. Witold Tatkowski, Radiation Safety Officer, in the Office of Radiation Safety. witoldtatkowski@brandeis.edu, or X6-4261
- For the animal care and use program, contact Dr. Scott Perkins, Attending Veterinarian (scott.perkins@tufts.edu). Questions concerning training and orientation to the Foster Animal Facility should be directed to Ms. Debra Goodwin, Animal Facility Director, Foster Comparative Medicine Services. dgoodwin@brandeis.edu or X6-2055

II. FOUNDATIONS OF BIOSAFETY

The foundations may be identified in several federal, state, and local regulations governing the use of recombinant/synthetic nucleic acids and microorganisms, human tissues and cell lines, and waste disposal. A brief summary of these documents or standards follows; additional information may be obtained by following the link to the appropriate website in the **Regulations and Resources** section.

National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, April 2019

These Guidelines specify safety practices for basic and clinical research involving recombinant and synthetic nucleic acid molecules, microorganisms, and viruses. Any institution receiving NIH funding for any research with recombinant/synthetic nucleic acids, regardless of the funding source (NIH-funded and non NIH-funded) for the particular project, must comply. This document defines the responsibilities of the NIH, the Institution at which the research is conducted, and the Principal Investigator. The Guidelines also require that an Institution establish an Institutional Biosafety Committee (IBC) for local review and oversight of recombinant or synthetic nucleic acid research, detailing the composition of the Committee and its function.

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition

This document presents a set of practices for handling biological materials, a reference for the construction or renovation of facilities, and a resource for developing new biosafety policies. Although the recommended practices, equipment, and facility safeguards are self-described as advisory in nature, this information represents recommended best practices in promoting a safe working environment and is considered mandatory by the regulatory committees of many academic institutions. Compliance with the BMBL, 6th Edition is a regulatory requirement for work involving select agents and toxins.

Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens (BBP) Standard

This Standard covers occupational exposure to human blood and other potentially infectious materials, including human and non-human primate tissues and cells, which could result in the transmission of bloodborne pathogens (HIV 1, Hepatitis B and C viruses, and additional infectious agents). OSHA recommends the use of universal precautions as a method of infection control and specifies a combination of engineering controls/safety equipment, work practices, and training to reduce the risk of infection.

Commonwealth of Massachusetts Medical Waste Disposal

These regulations include requirements for regulated medical or biological waste storage, treatment, and disposal, sharps collection and disposal, and approved disinfection methods.

III. REGULATIONS & RESOURCES

A. Regulations

Several federal, state, and local agencies regulate the use of recombinant/synthetic nucleic acids and biological agents, as well as waste derived from those research activities. A list of the responsible agencies and the corresponding web addresses is provided below:

National Institutes of Health
Biosafety
NIH Guidelines, April 2019
http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

Centers for Disease Control and Prevention
Biosafety
Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition
Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition | CDC Laboratory Portal | CDC

Arthropod Containment Guidelines (Version 3.2)
The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene
http://www.sc.edu/ehs/Biosafety/Arthropod%20Containment%20Guidelines%20(ACME).pdf

United States Department of Labor
Occupational Safety and Health Administration
Bloodborne Pathogens Standard 29 CFR 1910.1030
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051
Bloodborne Pathogens Standard 29 CFR 1910.1030 – Interpretations
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21010&p_text_version=FALSE

CDC/USDA Select Agent Program
http://www.selectagents.gov/
Select Agents and Toxins List
http://www.selectagents.gov/SelectAgentsandToxinsList.html

National Institutes of Health
Biosecurity
Dual Use Research of Concern
http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern

State of Massachusetts
Health and Human Services
http://www.mass.gov/eohhs/gov/departments/dph/
Medical Waste
http://www.mass.gov/eohhs/gov/departments/dph/programs/environmental-health/comm-sanitation/medical-waste.html
Medical Waste Regulation
http://www.mass.gov/eohhs/docs/dph/regs/105cmr480.pdf

City of Waltham
Department of Health
http://www.city.waltham.ma.us/health-department

B. Resources

A list of websites which provides information on biosafety and biosecurity, the availability and authentication of cell lines, and the characteristics of various infectious agents follows below.

National Institutes of Health
Office of Biotechnology Activities
http://osp.od.nih.gov/office-biotechnology-activities?IBCIIBCindexpg.htm

ATCC (American Type Culture Collection)
Cell Biology Collection, Microbiology Collections, Learning Center
http://www.atcc.org/en.aspx

Public Health England
Culture Collections
Cross-Contaminated and Misidentified Cell Lines
https://www.phe-culturecollections.org.uk/services/celllineidentityverification/misidentifiedcelllines.aspx

Public Health Agency of Canada
Laboratory Biosafety and Biosecurity
http://www.phac-aspc.gc.ca/lab-bio/index-eng.php

ViralZone SIB, Swiss Institute for Bioinformatics
http://viralzone.expasy.org/

American Biological Safety Association (ABSA International)
http://www.absa.org/

Brandeis University
Office of Research Administration
Institutional Biosafety Committee
http://www.brandeis.edu/ora/compliance/ibc/index.html
Applications and Forms
http://www.brandeis.edu/ora/compliance/ibc/forms/index.html

IV. RESPONSIBILITIES UNDER THE NIH GUIDELINES
INCLUDING REGISTRATION OF RESEARCH

A. Responsibilities

The NIH Guidelines detail procedures and practices for the containment and safe performance of research involving recombinant and synthetic nucleic acid molecules, including research involving genetically modified plants and animals, and human gene transfer. Any institution that receives NIH funding for research involving recombinant/synthetic nucleic acid molecules and/or microorganisms conducted at or sponsored by that institution must comply with the NIH Guidelines. Researchers at institutions that are subject to the NIH Guidelines must comply with the requirements, even if their own projects are not funded by NIH. Adherence to the Guidelines is mandatory and important because of the prescribed biosafety and containment measures.

The role and responsibilities of the Principal Investigator, the Institution, and the Institutional Biosafety Committee (IBC) as described in the Guidelines are briefly outlined here so as to familiarize PIs, laboratory researchers, IBC members, and administrators with their respective positions in the regulatory framework. Those responsibilities delegated to the Biosafety Officer, the NIH Director, Recombinant DNA Advisory Committee and Office of Science Policy are not enumerated in this section.

As extracted from the NIH Guidelines and summarized in the Investigator Educational Brochure, the responsibilities of the Principal Investigator (PI) shall include, but are not limited to:

- Determining whether the research is subject to Section III-A, III-B, III-C, III-D, or III-E of the NIH Guidelines (research which requires IBC approval prior to initiation).
- Proposing physical and biological containment levels in accordance with the NIH Guidelines when registering research with the IBC.
- Proposing appropriate microbiological practices and laboratory techniques to be used for the research.
- Submitting a research protocol to the IBC for review and approval.
- Remaining in communication with the IBC throughout the conduct of the project.
- Making available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken.
- Instructing, training and supervising laboratory staff in techniques required to ensure safety and procedures for dealing with accidents.
- Reporting any significant problems to the Biosafety Officer (where applicable), Animal Facility Director (where applicable), IBC, and NIH Office of Science Policy within 30 days.
- Petitioning the NIH Office of Science Policy, with notice to the IBC, for proposed exemptions from the NIH Guidelines.
- Seeking NIH Office of Science Policy's determination regarding containment for experiments that require case-by-case review.

Each institution is responsible for ensuring that research involving recombinant/synthetic nucleic acids is conducted in conformity with the provisions of the NIH Guidelines. In fulfilling this responsibility, the Institution shall:

- Establish and implement policies for the safe conduct of research subject to the NIH Guidelines.
- Establish an Institutional Biosafety Committee.
- Appoint a Biosafety Officer, if necessary.
- Appoint an individual with expertise in plant, plant pathogen, or plant containment to the IBC, if necessary.
- Appoint an individual with expertise in animal containment to the IBC, if necessary.
- Ensure that the institution complies with all regulations and obtains all authorizations for recombinant and synthetic nucleic acid research involving human participants.
- Assist and ensure compliance with the NIH Guidelines by Principal Investigators.
- Ensure appropriate training for IBC members and staff, the Biosafety Officer, PIs, and laboratory staff.
- Determine the necessity for health surveillance of personnel.
- Report any significant accidents, incidents or violations to the NIH Office of Science Policy within 30 days, unless a report has already been filed by the PI or IBC.

The Institutional Biosafety Committee (IBC), with regard to membership and functions, shall:

- Be comprised of no fewer than five members so selected that they have the experience and capability to assess the safety of recombinant or synthetic nucleic acids molecule research.
- Have at least two members that shall not be affiliated with the institution and who represent the interest of the surrounding community.
- Include one individual with expertise in either plant or in animal containment principles.
- Include a mandatory Biosafety Officer when conducting research at Biosafety Level 3 or 4.
- For research involving human participants, have adequate expertise and training, ensure that all questions have been answered by the PI, that no participant has been enrolled until the NIH registration process has been completed.
- Review recombinant or synthetic nucleic acid molecule research at or sponsored by the institution.
- Notify the PI of the results of the IBC's review.
- Set containment levels for experiments involving whole plants or whole animals.
- Periodically review recombinant or synthetic nucleic acid research conducted at the institution.
- Report any significant problems or violations of the NIH Guidelines to the appropriate Institutional Official and NIH Office of Science Policy within 30 days.

Although not explicitly stated within the NIH Guidelines, the responsibilities of laboratory personnel can be reasonably assumed to include the following:

- Comply with safety recommendations for the work being performed.
- Receive training in appropriate laboratory techniques and be knowledgeable of the nucleic acids and/or biological agents under manipulation and the associated risks.
- Report accidents or injuries to the PI or Supervisor.

B. Submitting a Registration/Protocol for Research

The NIH requires all laboratories working with recombinant and synthetic nucleic acids to register with their local Institutional Biosafety Committee. The Guidelines describe six categories of experiments, with the categories distinguished from one another by the potential hazard of the work. As first in the list of responsibilities for the Principal Investigator given above, the PI must determine the category or categories under which the proposed experiment(s) fall. The PI must then make an initial determination of the required physical and biological containment levels; the Handbook section concerned with **Risk Assessment** provides information on this determination, while Section II-B of the NIH Guidelines discusses issues to be considered in a further refinement of the containment level for the proposed work. The forms for the registration of research at Brandeis University, and the corresponding instructions for completion, may be found under the Institutional Biosafety Committee Applications and Forms website: http://www.brandeis.edu/ora/compliance/ibc/forms/index.html

A summary of the six classifications of experiments involving recombinant and synthetic nucleic acids to assist with the registration follows, along with the relevant sections of the NIH Guidelines and the corresponding level of review.

1. Experiments that compromise the control of disease agents through deliberate transfer of a drug resistance trait; **NIH Guidelines Section III-A**.

   Relevant information should be sent to the NIH Office of Science Policy, and NIH Director and Institutional Biosafety Committee review and approval is required before initiation.

2. Experiments involving the cloning of toxin molecules with an LD50 of less than 100 nanograms per kilogram of body weight; **NIH Guidelines, Section III-B**.

   These experiments require both NIH Office of Science Policy and IBC review and approval before initiation.

3. Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participants (human gene transfer); **NIH Guidelines, Section III-C**.

   These experiments require IBC and all other applicable institutional (IRB) and regulatory authorizations and approvals before research can be initiated.

4. Experiments which require IBC approval before initiation; **NIH Guidelines, Section III-D**.

   A. Experiments involving the introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2, 3, or 4, or Restricted Agents. Experiments using whole animals with such agents will usually be conducted at ABSL 2 (animal biosafety level), ABSL 3 or ABSL 4 containment, respectively.

   B. Experiments in which DNA from a Risk Group 2, 3, or 4, or Restricted Agent is cloned into a prokaryotic or lower eukaryotic host-vector system.

   C. Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems. Experiments involving the use of infectious or defective Risk Group 2, 3, or 4 viruses may be conducted under BSL 2, 3, or 4 containment, respectively.

Note: recombinant or synthetic nucleic acid molecules, or nucleic acid molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus are considered defective and may be used in the absence of helper under the conditions specified in Section III-E.

D. Experiments involving whole animals in which the animal's genome has been altered by the stable introduction of recombinant or synthetic nucleic acids molecules, or nucleic acids derived therefrom, into the germline (transgenic animals), and

Experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals. These experiments, other than using viruses that are only transmitted vertically, must be conducted in a minimum containment of BSL 2 or ABSL 2. Recombinant or synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate and propagated under conditions comparable to BSL 1 or ABSL 1 and appropriate to the organism under study. Animals that contain sequences from viral vectors which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in the animal may be propagated under physical containment comparable to BSL1 or ABSL 1.

For experiments involving recombinant or synthetic nucleic acid molecule-modified Risk Groups 2, 3, 4, or restricted organisms, the investigator must demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with animal of plant pathogens.

For experiments involving recombinant or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals and including transgenic animals, not covered by Risk Group 2, 3, 4, and Restricted Agents (Experiments Using Human or Animal Pathogens), the appropriate containment level will be determined by the Institutional Biosafety Committee.

E. Experiments involving more than 10 liters of culture.

The appropriate containment will be determined by the IBC.

F. Experiments with influenza viruses generated by recombinant or synthetic methods (e.g., generation by reverse genetics of chimeric viruses with reasserted segments, introduction of specific mutations) will be conducted at the biosafety level containment corresponding to the Risk Group of the virus that was the source of the majority of segments in the recombinant or synthetic virus.

5. Experiments the require IBC notice simultaneous with initiation; **NIH Guidelines, Section III-E**.

Experiments not included in Sections III-A, III-B, III-C, and III-E are considered in this Section; e.g., experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes may be conducted at BSL 1.

A. Recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated and maintained in tissue culture cells under BSL 1 containment.

B. The generation of transgenic rodents where the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, where only BSL 1 containment is required is covered in this Section (experiments that require BSL-2, -

3. And -4 containment are covered under Section III-D4, Experiments Involving Whole Animals).

Experiments involving the breeding of certain BSL 1 transgenic rodents are exempt under the following Section III-F.

6. Exempt Experiments; **NIH Guidelines, Section III-F**.

A. Experiments involving synthetic nucleic acid moleculesthat can neither replicate nor generate nucleic acids that can replicate, that are not designed to integrate into DNA, and do not produce a toxin with an LD50 of less than 100 nanograms per kilogram body weight are exempt.

B. Included are those nucleic acids not found in organisms, cells, and viruses, and have not been physically modified to render them capable of penetrating cell membranes.

C. Those nucleic acids that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

D. Experiments utilizing nucleic acids obtained entirely from a prokaryotic host, including its indigenous plasmids and viruses, when propagated only in the respective host (or a closely related strain of the same species).

E. Experiments utilizing nucleic acids obtained from a eukaryotic host, including its chloroplasts, mitochondria, and plasmids (but excluding viruses) when propagated only in that host or in a closely related strain of the same species.

F. Experiments using nucleic acids that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.

G. The breeding, purchase, and transfer of certain BSL 1 transgenic rodents.

H. Those nucleic acids that do not present a significant risk to health or to the environment, as determined by the NIH Director following appropriate notice and opportunity for comment.

While the term “exempt” indicates that registration is not required if the research is not covered by any of the Sections III-A to III-E of the NIH Guidelines, Brandeis University requires that all research, exempt or non-exempt, be registered with the IBC. The rationale behind this approach is that for biosafety to be meaningful, the practices, procedures, and containment measures must be observed by all investigators at an institution. The exempt work can usually be performed at Biosafety Level 1.

V. BIOSAFETY PRINCIPLES

A. Containment

An important element of biosafety is the containment of harmful biological agents to eliminate or reduce exposure to laboratory workers, individuals in the surrounding area, and the outside environment. Containment may be further divided into two categories: primary and secondary. Primary containment is intended to protect laboratory personnel and the immediate laboratory environment from exposure, and is achieved through the use of safety equipment, personal protective equipment, and good microbiological technique. Secondary containment involves protection of the environment outside the laboratory, and is effected through facility design and engineering controls (e.g. processes for ventilation, noise absorption). The appropriate degree of containment for a particular research activity results from the proper use of three components:

1. **Safety Equipment**
2. **Facility Design**
3. **Laboratory Practice and Technique**

1. Safety Equipment

Safety equipment includes the biological safety cabinet, autoclave, and other personal protective equipment designed to minimize exposure to hazardous biological agents.

Biological Safety Cabinet (BSC)

A BSC provides protection to personnel working within the cabinet, the immediate laboratory environment, and the research materials. The safety cabinet is most effective at minimizing exposure when properly used and maintained (see **Attachment A** for detailed information on effective use of a BSC). The BSC should be inspected and certified on a yearly basis.

Autoclave

Moist heat in the form of steam under pressure is widely used and a dependable method for sterilization. The moist heat works its effects through the irreversible coagulation and/or denaturation of enzymes and structural proteins of an organism. The proper operation of an autoclave and the appropriate cycle and time settings for a given load of items is given in **Attachment B**.

Sharps Containers

When dealing with needles or sharp instruments:

- Needles must not be recapped, bent, sheared, broken, or removed from the disposable syringe before discarding.
- Used disposable needles and syringes must be placed in a puncture-resistant sharps container for disposal. The container must not be filled to greater than 75% full/above the designated fill line on the label.

As of June 6, 2016, the Brandeis University-specific policy is that all components of a syringe (plunger, needle, and cap) must be disposed of in a sharps container. No part of a syringe should be placed in the regular trash or a glass trash box, even when the plunger is the sole part without any attached needle.

- Non-disposable sharps, such as surgical scissors, fine forceps, and reusable needles, must be placed in a puncture-resistant container for transport to a processing area for further cleaning and sterilization.
- Broken glassware must not be handled directly; rather, the glassware must be removed using a brush and dustpan, or forceps. Plasticware should be substituted for glassware whenever possible.

Personal Protective Equipment

Personal protective equipment (PPE), such as lab coats, gloves, safety eyewear, and a respirator is used to supplement the containment provided by safety equipment. Personal protective equipment is considered a primary barrier and will reduce the likelihood of infection when properly used.

Laboratory Coats

Laboratory coats are used to protect personnel against hazardous materials and to prevent the spread of those materials outside the lab (as long as the coats are retained within the lab). In choosing the correct lab coat, an assessment of the types of materials that are in use in the laboratory, encompassing chemicals, flammable materials, radioisotopes, and biological agents, is necessary. Coats may be either reusable or disposable, and may be composed of materials such as fire-resistant cotton, 100% cotton, or a synthetic/cotton blend. Wearing lab coats is considered to be standard microbiological practice for BSL 1 and 2 laboratories, and the coats are to be left in the laboratory and not taken home to be washed.

Disposable laboratory coats are recommended for use within the Brandeis University BSL 2+ facility, where viral vectors are produced and/or aliquoted. These coats are also to remain in the facility, and have the advantage of being deposited directly to solid waste should the coat become contaminated.

Gloves

Gloves are available in a variety of materials, such as latex, nitrile, polyethylene, and polyvinyl chloride, and are used as protection against chemicals, radioactive materials, and infectious agents. Selection of the type of glove to be used is based on the nature of the material and the conditions of the manipulation, encompassing chemical, radiological, or biological hazards, the use of sharps, extremes in pH and/or temperature.

When in use, gloves should always be:

- Checked for rips or punctures,
- Replaced with new ones when visibly worn, when contaminated through a spill or splash, and
- Removed before touching common objects such as door handles or elevator buttons.

Latex gloves provide good protection against biological and water-based materials, including human blood and body fluids. These gloves do not generally provide adequate protection against chemicals and against organic

solvents in particular. Furthermore, puncture holes are difficult to detect and the gloves may aggravate latex allergies. Nitrile gloves are very good for general use, and are also effective against biological hazards. They are an excellent alternative to latex, providing similar dexterity and increased chemical resistance to solvents, oils, greases, and some acids and bases.

Controlling the spread of hazardous biological materials or chemicals requires that gloves be removed upon exiting a biosafety cabinet or before touching non-contaminated laboratory areas and equipment. Gloves should be carefully removed, from the inside out, so as to minimize contact between the contaminated exterior of gloves and bare skin. The proper procedure for removing gloves is illustrated below (from https://www.cdc.gov/vhf/ebola/pdf/poster-how-to-remove-gloves.pdf):

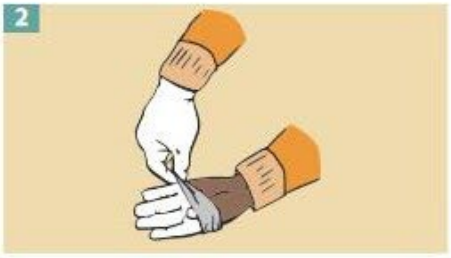
How to Remove Gloves

To protect yourself, use the following steps to take off gloves

1

With both hands gloved, grasp the outside of one glove at the top of your wrist, being careful not to touch your bare skin.

2



Peel off this first glove, peeling away from your body and from wrist to fingertips, turning the glove inside out.

3

Hold the glove you just removed in your gloved hand.

4

With your ungloved hand, peel off the second glove by inserting your fingers inside the glove at the top of your wrist.

5

Turn the second glove inside out while tilting it away from your body, leaving the first glove inside the second.

6

Dispose of the gloves safely. Do not reuse the gloves.

7

Clean your hands immediately after removing gloves and before touching any objects or surfaces.

One Glove Rule

Gloves must be worn when there is the potential of coming into contact with hazardous materials, whether radioactive, chemical, or biological. In turn, all personal protective equipment must be removed prior to exiting the laboratory so as to keep common areas (restrooms, elevators, offices, card swipes, stair rails, and door handles) free from contamination.

The One Glove Rule was established to lessen the chance of contamination and exposure outside the laboratory. If material is to be transported from the lab through common areas, the best practice is to use a package that allows handling of the outside without gloves yet contains the contents if dropped. A double-containment system (incorporating nested leak-proof, sturdy containers surrounded with absorbent material) is recommended for transporting materials from one location to another within the University. However, under certain circumstances the use of such a containment system may not be feasible, so that transport will then involve the use of an ungloved hand to touch common surfaces and a gloved hand to carry the item.

Eye and Face Protection

Safety glasses, goggles, and face shields provide eye and face protection against splashes of chemical and biological materials, as well as radiant energy such as lasers and ultraviolet light sources. Laboratory environments and the associated research vary widely, so the type of eyewear that is used should be appropriate to the risks inherent in the laboratory. Typical prescription eyeglasses are not safety glasses and do not provide adequate eye protection for laboratory work.

Safety glasses provide adjustability and a comfortable fit, with some versions capable of being worn over prescription glasses, although this type of eyewear offers limited splash protection to the face. Goggles, when fit against the face, provide protection when there is a moderate risk of splashes or sprays. A set of goggles, indirectly vented, will allow for the passage of air between the exterior and interior of the goggles while protecting against splashes and projectiles to the eyes. A face shield is recommended if there is a high risk of a splash, and it effectively protects the entire face when worn in conjunction with safety glasses or goggles.

Respiratory Protection

Work with hazardous materials that can give rise to aerosols should generally be performed in a biological safety cabinet (for aerosolized microorganisms) or a chemical fume hood (for chemical vapors and gasses). This protection from airborne exposure can reduce the need for a respirator. However, certain work requirements and circumstances may require the use of respiratory protection.

Whenever laboratory personnel are required to wear any respirator (including the N-95 particulate filtering face piece respirator) to prevent exposure to hazardous materials, prior approval from the Department of Environmental Health and Safety is necessary. The individual must be fit-tested, and trained prior to using

respiratory protection. In the case where the use of a respirator is voluntary, the individual utilizing the respirator must be medically evaluated and trained. Questions regarding the use of respirators may be addressed to Mr. Andrew Finn of the Department of Environmental Health and Safety, X6-4262, or afinn@brandeis.edu, and respirator fit-tests may be scheduled by contacting Debra Goodwin, X6-2055, or dgoodwin@brandeis.edu.

2. Facility Design

Facility design contributes to the protection of both personnel within the laboratory, incorporating features that enhance the functions provided by safety equipment, and members of the outside community. The design will factor in the risk of transmission of specific agents, tailoring the construction of the facility to exposure risks of the specific agents and the appropriate biosafety level for manipulation (e.g. a specialized ventilation system for an agent capable of being dispersed through aerosols). Controlled access into the rooms and the physical separation of laboratories, a ventilation system operating a negative pressure within the facility, and the availability of an autoclave are several features that protect personnel within the laboratory as well individuals outside.

3. Laboratory Practice and Technique

This component is perhaps the most important of containment, and consists of:

a. Strict adherence to good microbiological practices.
b. Knowledge of infectious agents and associated risks.
c. Development of standard operating procedures.

Good Microbiological Practices

1. Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents.
2. Use personal protective equipment (PPE), such as lab coats, gloves, and safety eyewear.
3. Decontaminate work surfaces and equipment before and after use, and immediately after spills.
4. Avoid touching your face with gloved hands.
5. Manipulate infectious fluids carefully to avoid spills and the production of aerosols.

For the Prevention and Containment of Aerosols and Droplets:
The handling of liquids or powders is likely to generate aerosols or droplets. Activities such as vortexing, pipetting, and centrifuging may produce aerosols that are small enough to be inhaled. Procedures involving infectious material should be performed inside a biological safety cabinet whenever possible. A fume hood should be used whenever hazardous materials in the form of volatile liquids or fine powders are weighed or mixed.

Pipetting:
A mechanical pipetting device is always to be used. Pipettes should be drained gently with the tip against the inner wall of the receiving vessel and liquid gently dispensed from the pipette.

Blending:
A safety blender that has leak proof bearings and a tight-fitting lid with a sealable gasket should be used. The blender should be placed inside a safety cabinet when blending material containing infectious agents.

Centrifugation:
The potential for contamination is high if any liquid and aerosol are released during centrifugation. Sealed centrifuge buckets should be used to prevent the release of any material. Rotors containing infectious agents must be loaded and unloaded within a biosafety cabinet. Small bench top centrifuges can be placed inside a biosafety cabinet to contain aerosols when infectious materials are used.

6. Limit the use of needles, syringes, and other sharps to those procedures for which there are no alternatives. Dispose of sharps in leak- and puncture-proof containers.
7. Do not eat, drink or store food in the laboratory.

Knowledge of Infectious Agents and Associated Risks/Biological Hazard Information

Personnel working with a biological agent must have access to the relevant information, be knowledgeable of the hazards associated with the agent, and be trained to safely handle and dispose of the material. Foremost among the sources of information is the laboratory-specific Research Registration prepared by the Principal Investigator which details the nucleic acids and/or microorganisms to be used and the handling and disposal procedures to be employed. In addition, the following online sources provide hazard information on a variety of biological agents (Prokaryotic/Fungal/Viral):
1. The Biosafety in Microbiological and Biomedical Laboratories, 6th Edition provides summaries on a number of specific bacterial, fungal, parasitic, and viral agents and the recommended biosafety levels and work practices for these agents.
2. The Public Health Agency of Canada offers Pathogen Safety Data Sheets that describe the hazardous properties of a large number of pathogens and practices for safe manipulation and decontamination.
3. The American Biological Safety Association maintains a database for the Risk Group Classification of several infectious agents.

Development of Standard Operating Procedures (SOPs)

This Handbook, in combination with the BMBL, 6th Edition and NIH Guidelines, is meant to provide overall standard operating procedures for work with nucleic acids and biological agents. However, because these procedures cover relatively general topics, individual laboratories should develop specific SOPs that address the biosafety concerns of that laboratory. Laboratory-specific SOPs should describe the safe manipulation of microorganisms, specific exposure control methods, and specific decontamination and waste handling procedures.

Transport of Biological Materials

BSL 2 Infectious agents/viral vectors must be transported between laboratories/buildings within the University using a double containment system. The system should consist of three components:

1. A leak-proof primary container (vial or tube) holding the microorganism/biological agent,
2. A leak-proof secondary receptacle, and
3. An outer, tertiary, leak-proof package.

Both the secondary and tertiary containers must be durable as well as leak-proof so as to prevent the accidental release of the biological agent. Absorbent material may be placed around the primary container in an amount sufficient to absorb all fluid in the primary container. In addition, a frozen ice pack may be incorporated around the primary container and located within the secondary container in order to keep the contents frozen, if desired. The surface of the ice pack can subsequently be disinfected after use, if necessary. Additional absorbent material is placed between the secondary and outer containers so as to absorb all remaining biological material in the event of breakage or leakage of the internal sample.

B. Risk Assessment (Risk Groups and Biosafety Levels)

Risk assessment represents a process by which risks involving biological (and chemical and physical) hazards are identified, evaluated for likelihood and severity, and then managed and monitored (for a more detailed description see https://www.cdc.gov/safelabs/resources-tools/bio-risk-assessment.html). This process, illustrated in **Figure 1**, should be applied before any new research is undertaken, and applied again whenever there is a change in work practices, personnel, and equipment. The principal investigator (PI) will usually initiate a risk assessment of the materials and procedures used in conducting the laboratory's research, with institutional leadership, safety professionals (on an ad hoc basis, if deemed necessary), facility managers, and the IBC also contributing. As previously mentioned, the PI is required to determine the physical and biological containment practices appropriate to the research planned for the laboratory, and to draw up and make available to the staff those safety procedures to be used with the research materials. Briefly, the steps taken during this process involve:

1. The identification of hazards associated with the materials being used and the risks (e.g. incidents involving injury, exposure, and infection).
2. The evaluation of those risks in terms of likelihood (involving factors that affect whether or not the incidents happen), followed by consequences (including factors that affect the severity of those incidents). Based on these evaluations, the various risks are prioritized and a determination is made by the laboratory as to what risks are acceptable and which are unacceptable. It is important to note that while certain risks can be reduced (e.g. either by the use of an attenuated micro-organism or of a related non-pathogenic strain), the risk may not be completely eliminated.
3. The creation of a risk mitigation plan, involving the elements of containment such as safety equipment, facility design and its construction, and laboratory practice and technique.
4. The actual implementation of the plan.
5. The evaluation of the effectiveness of the plan, an evaluation that should be performed routinely. The introduction of new materials and procedures into the laboratory should also trigger a new round of assessment, moving through the aforementioned steps once again.

In evaluating the risks (Step 2, above), the risk group of a microorganism may provide relevant information. The risk group is a classification based on the intrinsic characteristics of an agent, such as its ability to cause disease, the severity of that disease, and the availability of treatments (see **Table 1**, and the NIH Guidelines, April 2019, Appendix B for the classification of specific organisms). The biosafety level will, in general, correlate with the

risk group; see **Table 1** and **Figure 1.** The risk group, however, is but one factor to be considered; other factors include possible modes of transmission, the nature of the material being used (e.g., genomic nucleic acid versus intact virus), and the experience of research personnel. Specific circumstances, such as the use of an attenuated virus or an oncogene in a viral vector, may dictate that the recommended safety level be lowered or raised. The risk group of a derived vector is generally lower than that of the respective wild-type virus as long as the genetic modification(s) lead to the attenuation of the virus. However, the properties of a transgene must always be considered when assigning the use of a particular vector to a biosafety level.

Table 1. Classification by Risk Group and Corresponding Biosafety Level

Risk Group	Description and Examples	Biosafety Level
Risk Group 1 (RG1)	**Agents that are not associated with disease in healthy adult humans** (*E. coli*, *S. cerevisiae*, Baculoviruses, Adeno-associated virus, AAV vectors)	**BSL 1**
Risk Group 2 (RG2)	**Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available** (*S. aureus;* Adenovirus; Adenoviral vectors; lentivectors based on HIV 1, SIV 1; human and non-human primate tissues, cells, cell lines)	**BSL 2**
Risk Group 3 (RG3)	**Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available; high individual risk but low community risk** (*M. tuberculosis*, HIV 1, SARS-CoV-1, SARS-CoV-2)	**BSL 3**
Risk Group 4 (RG4)	**Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available; high individual risk and high community risk** (Ebola virus, Marburg virus)	**BSL 4**

(Modified from the *NIH Guidelines, April 2019*, p. 42)

The four biosafety levels, as presented in the BMBL, 6th Edition, describe the safety equipment, facility design, and laboratory practices and techniques for work with specific infectious organisms. The safety level recommended for a microorganism, representing conditions under which the agent can be safely handled, should be appropriate for the particular procedures used, for any documented or suspected transmission route, and for the overall activity of the laboratory. Additional information on the safety equipment, laboratory organization, and practices pertinent to each biosafety level is summarized in **Table 2**, and described more fully in the BMBL, 6th Edition.

Distinct from the biosafety levels established by the BMBL, 6th Edition, a BSL 2+/BSL 2-enhanced facility may be designed by an institution in response to specific research that would benefit from the addition of certain BSL 3 safety elements to the standard BSL 2 facility. The highest level of research operating at Brandeis University is

BSL 2+.

In addition, a set of four animal biosafety levels, ABSL 1 through 4, have been assigned to the use of vertebrate animals experimentally infected with biohazardous agents or naturally harboring zoonotic infectious agents (Section V, BMBL, 6th Edition). As a general principle recognized by the BMBL, the biosafety level recommended for working with infectious agents *in vivo* is considered comparable to the *in vitro* level. This recommendation also factors in a risk assessment based on an animal protocol, and on the presence of an occupational health and safety program at the institution. The four biosafety levels represent four combinations of equipment, facilities, and practices for research involving animals carrying an infectious microorganism or for any experiment that may benefit from these types of containment. Again, the ABSL 1 to 4 levels are applicable to experiments with animals infected with BSL 1 to 4 agents, respectively, and provide for increasing levels of protection to research personnel and the surrounding environment.

Appendix E of the BMBL, 6th Edition furthermore covers the use of "arthropods of public health importance" in research. Four levels of containment (Arthropod Containment Levels 1 to 4) have been advanced, similar to the BSLs described above, but also factor in hazards associated with the accidental escape of arthropods with a phenotype such as insecticide resistance and with genetically modified arthropods. For a more complete description of the practices, safety equipment, and facilities recommended for arthropod research, the Arthropod Containment Guidelines (ACG Version 3.2) (VBZ-2018-2431-ver9-Bartholomay_5P 152..173 (nih.gov)) should be consulted.

Biosafety in Field Research

Fieldwork represents another component of the research and teaching activities at the University, performed by faculty, students, and staff at either on- or off-campus locations. A thorough planning and preparation of field studies, based on an understanding of the potential environmental, physical, and biological hazards, should be performed prior to starting the work. Research involving animals would require registration with and approval from the Brandeis University Institutional Animal Care and Use Committee (IACUC), while research involving microorganisms and recombinant or synthetic DNA and RNA would need to be registered and approved by the Institutional Biosafety Committee (IBC). In order to assist in the development of policies and procedures for the safe conduct of fieldwork, advice by the Guidance on Health and Safety in Fieldwork, 2011, (http://www.ucea.ac.uk/en/publications/index.cfm/guidance-on-health-and-safety-in-fieldwork), is offered to institutions of higher education regarding several important preliminary planning steps. The guidelines cover a variety of aspects to be considered, including the roles and responsibilities of institutional administrators, fieldwork leaders, and researchers, as well as considerations on risk assessment, emergency responses, and researcher competence and training.

While a field site differs from a more permanent structure such as a laboratory and is not necessarily concerned with the escape of animals or other organisms into the surrounding environment, the specimen collected may constitute a biohazard itself or carry an infectious agent. Among the potential biological hazards associated with field work are exposure to poisonous plants, venomous animals, animal blood, tissues and body fluids, and vector-borne and zoonotic diseases. The type of containment, safety equipment, and procedures used in the field should be based on the particular biohazards associated with the animal, plant, or microorganism being collected. The Safe Precautions to Avoid Zoonotic Disease from Wildlife; Quick Reference Guide by the National Park Service

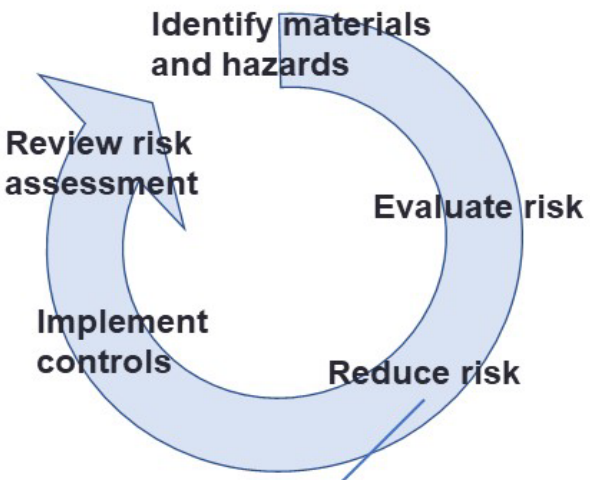
offers four scenarios (ranging from a low risk activity to one with a high risk of zoonotic disease) and details the appropriate PPE and work practices that should be in effect in each case.

The National Park Service's Quick Reference Guide also offers guidelines regarding standard precautions, pathogen exposure routes, and the application of PPE in avoiding exposure to zoonotic diseases. In addition, several safety procedures may be employed against mosquitos, ticks, and fleas in order to avoid bites, stings, and vector-borne diseases. An excellent web resource covering tick identification and protection against tick exposure may be found at the TickEncounter Resource Center (http://www.tickencounter.org/). In general, the safety precautions include the following:

- Use of standard precautions when working with wildlife or potentially infectious materials, such as wearing gloves, thorough handwashing after glove removal, disinfection of soiled equipment and contaminated environmental surfaces, and avoiding needlestick wounds, bites, scratches and other physical injuries.
- Avoid conducting fieldwork during peak insect activity times (usually during the early morning and evening hours) in habitats favorable to insects (wetlands, streams, etc.).
- Wear a long-sleeved shirt and long pants made of tightly woven materials. Tuck pants into boots or socks.
- Wear light-colored clothing in the field so that ticks and other insects can be spotted and removed.
- Use insect repellants containing compounds such as DEET on exposed skin and Permethrin on clothing only. The reference guide Safe Precautions to Avoid Zoonotic Disease from Wildlife lists several repellents for mosquitos and ticks, their effective concentrations and contraindications to their use.
- Tie back hair and wear a hat.
- Whenever possible, fieldwork should be done in teams of at least two people. This "buddy" system allows each team member to inspect the other for attached ticks and other insects at the end of each sampling excursion.
- Avoid shrubbery and stay on the widest part of any path.
- If you are stung or bitten, wash the wound with soap and water, apply an antiseptic, and cover the wound with a bandage or clean dressing. Carefully remove stingers from the skin by using tweezers and then clean and dress the wound.

The method of preservation of any collected arthropods will depend on the type of insect and the subsequent analyses to be conducted. Whether the samples are mosquitos, ticks, or moths, the method of preservation should be appropriate to the studies to be performed, which may range from morphological examinations to the extraction of nucleic acids. The document Preserving Insects and Related Arthropods, Rondon, S. and Corp, M., Oregon State University (http://extension.oregonstate.edu/umatilla/sites/default/files/PRESERVING_INSECTS.pdf) presents a variety of methods, suggesting a range of alcohols (70% to 95%), acetone, dry preservation, and refrigeration or freezing depending on the particular insect and research goal.

Figure 1 Risk Assessment



Appropriate degree of containment for a particular activity.

- Safety equipment
- Facility design and construction
- Laboratory practice and technique

RG1 Not associated with disease. **BSL 1**
E. coli K-12, AAV, AAV vectors

RG2 Disease rarely serious; interventions often available. **BSL 2**
AdV; vectors derived from HIV 1 (lentivectors); human tissues, body fluids, and cell lines

RG3 Disease serious or lethal; interventions may be available. **BSL 3**
HIV 1, M. tuberculosis

RG4 Disease serious or lethal; interventions not usually available. **BSL 4**
Marburg virus

(The microorganisms and materials listed above are representative of the Risk Group, and do not constitute a complete list)

Figure 1. Risk assessment steps:

1. Identify the hazards associated with each material and each procedure, and then the risks. First ascertain what, where, and how the work is occurring and who is doing the work, and then determine what could go wrong in working with the material.
2. Evaluate the risks; determine the likelihood and severity of the risk, and if the risk is acceptable or unacceptable. The risk group (a classification that describes the relative hazard posed by an infectious agent or toxin) is an important consideration, but not the sole one, used to determine the appropriate biosafety level at which an agent or toxin may be handled. Risk groups may correspond to biosafety levels at a first approximation, although it is possible that a risk assessment for a specific experiment may conclude that the agent can be handled at a lower or higher biosafety level. The likelihood of an incident (considering factors that affect whether an incident occurs) and the consequences (considering factors that affect the severity of the incident) should be determined. The risk should then be ranked based on severity and the laboratory should determine which of those risks are acceptable. Importantly, the reduction of risk may be achieved, but may not be eliminated.
3. Reduce risk by identifying appropriate containment actions (consider the appropriate equipment, lab design, and practices and procedures to be used for safe work).
4. Implement a risk mitigation plan.
5. Evaluate the effectiveness of the plan. Such evaluations should be performed routinely and whenever new materials and procedures are to be used.

Abbreviations: RG – risk group; BSL – biosafety level; AAV – adeno-associated virus; AdV – adenovirus; HIV 1 – human immunodeficiency virus 1.

Table 2. Summary of Recommended Biosafety Levels (Specifying Practices, Safety Equipment, and Facilities)

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls;[b] gloves;[b] full-body, air-supplied, positive-pressure suit[c]	Entry sequence; entry through airlock with airtight doors;[c] walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door, pass-through autoclave required

(From *BMBL 6th Edition*, p. 68-69)

VI. DECONTAMINATION

Decontamination, the process of removing or neutralizing hazardous chemicals or infectious organisms from personnel and equipment, is an important part of good microbiological practices and serves to protect laboratory personnel, other individuals in the immediate vicinity, and the environment. Sterilization and disinfection are two similar yet distinct components of decontamination; sterilization represents a physical or chemical process that destroys all microbial life, including spores, while disinfection is the physical or chemical means of destroying or inhibiting microbial growth with the exception of spores.

A. Chemical Disinfection

Chemical disinfectants are used to render contaminated work surfaces, biological safety cabinets, equipment, and other objects safe for further use. Laboratory workers should remember that there are hazards associated with these chemical disinfectants; inhalation and skin contact should be minimized, and eye contact must be avoided. Appropriate gloves, laboratory coat, and eyewear should always be worn when handling these chemicals.

There are several factors that affect the efficacy of the disinfectant, some of which are intrinsic to the organism and others which relate to the chemical and physical environment (from the CDC's Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008). Such factors include the:

1. Concentration of the disinfectant
2. Duration of exposure
3. Presence of organic/inorganic material surrounding the infectious agent
4. Presence of biofilms
5. Physical and chemical factors (pH, temperature, relative humidity, water hardness)
6. Number and location of microorganisms
7. Intrinsic qualities of the microorganism

Various microorganisms and viruses may be ranked according to their resistance to chemical disinfectants (from highest to lowest; **Figure 2**), based on their cellular structure, composition, and physiology. One general conclusion that may be derived from the Figure is that viruses lacking a membrane surrounding the outer capsid are more resistant to chemical disinfectants than enveloped viruses. There are, however, exceptions to this alignment. Several Pseudomonas species are sensitive to glutaraldehyde and formaldehyde, but growth in water and the subsequent formation of biofilms on the surface leads to resistance by the protected cells that can approach the resistance of bacterial spores to these same disinfectants. Prions are also highly resistant to

disinfectants, heat, ultraviolet radiation, ionizing radiation and formalin; however, prions can be deactivated by a combination of heat, chemicals, pressure, and time. Several of the more common disinfectants arrayed against various microorganisms in laboratory use are presented in **Table 3**. Starting with a particular disinfectant, the effective concentration to be used against targeted microorganisms and several accompanying notes relating to concentrations and shelf life are included.

Figure 2. Descending Order of Resistance to Antiseptics and Disinfectants

Prions
(Creutzfeldt-Jakob Disease, Bovine Spongiform Encephalopathy)

Coccidia
(Cryptosporidium)

Spores
(Bacillus subtilis, Clostridium difficile, Clostridium sporogenes)

Mycobacteria
(M. tuberculosis, M. avium, non-tuberculous mycobacteria)

Cysts
(Giardia)

Small non-enveloped viruses
(Poliovirus, Coxsackievirus, Rhinovirus)

Trophozoites
(Acanthamoeba)

Gram-negative bacteria (non-sporulating)
(Pseudomonas aeruginosa, Salmonella choleraesuis, Providencia spp.)

Fungi
(Trichophyton spp,, Cryptococcus spp., Candida spp., Aspergillus spp.)

Large non-enveloped viruses
(Enteroviruses, Adenoviruses)

Gram-positive bacteria
(Staphylococcus aureus, Enterococci)

Enveloped viruses
(HIV 1, HBV, HCV, Herpes simplex virus, CMV,
Respiratory syncytial virus, Hantavirus, Ebola virus)

(Adapted from Figure 1 of McDonnell, G and Russell, A.D. 1999. Clin. Microbiol. Rev. 12: 147-179, with additional microorganisms added from the University of North Carolina Biological Safety Manual, http://ehs.unc.edu/manuals/biological/10-4/)

Table 3. Summary of Common Laboratory Disinfectants

(The disinfectants listed below are most effective when prepared fresh)

Disinfectant	Effective Concentration	Targeted Microorganisms	Notes
Chlorine (Household bleach)	Minimum of 500 ppm	Wide Spectrum including: Vegetative bacteria Enveloped (lipid) viruses Non-enveloped (non-lipid) viruses Bacterial spores	-Corrosive -Inactivated by organic material -Does not have a long shelf life -Loses concentration with exposure to light and air, thus working solutions don't have a long shelf-life
Iodine	75 to 1600 ppm	Wide Spectrum including: Vegetative bacteria Enveloped viruses Non-enveloped viruses Bacterial spores	-Longer shelf life -Inactivated by organic material -Concentrated solutions are less effective as Iodine will be bind to itself or carrier molecules
Alcohol	70 to 85 percent	Vegetative bacteria Enveloped viruses Some non-enveloped viruses	-Ethyl Alcohol -Isopropyl Alcohol -Flammable -Evaporation rate due to vapor pressure may require repeated application
Quaternary Ammonium Compounds	0.1 to 2.0 percent	Vegetative bacteria Enveloped viruses	-Low-level disinfectant -Long shelf life -Inactivated by organic material -Modern formulations combined with other disinfectants are more effective

Disinfectant	Effective Concentration	Targeted Microorganisms	Notes
Phenol	1.0 to 5.0 percent	Vegetative bacteria Enveloped viruses Some non-enveloped viruses	-Long shelf life -Corrosive -Can leave residue -Tuberculocidal and fungicidal -Hard water can affect effectiveness
Formaldehyde	0.2 to 8.0 percent	Wide Spectrum including: Vegetative bacteria Enveloped viruses Non-enveloped viruses Bacterial spores	-Effective in presence of organic material -Sterilant, fixative -Diminished activity in colder temperatures -Non-corrosive -Suspected carcinogen, toxic at low levels
Hydrogen Peroxide	3.0 to 25.0 percent	Wide Spectrum including: Vegetative bacteria Enveloped viruses Non-enveloped viruses Bacterial spores	-Higher concentrations are sporicidal -Strong oxidizer, higher concentrations can burn skin

(From the Disinfection and Sterilization module, CITI Program. The chart had been previously adapted from Laboratory Safety Monograph 176 - A Supplement to the NIH Guidelines for Recombinant DNA Research, 1979)

B. Autoclaving

Moist heat in the form of saturated steam under pressure is widely used as a dependable method for sterilization. The exposure of any item to moist heat at 121°C (250°F) under pressure (at least 15 pounds per square inch; psi) for 15 to 30 minutes results in the destruction of all forms of microbial life. The mode of action is through the irreversible coagulation of enzymes and structural proteins, as moisture significantly affects the coagulation temperature and the temperature at which microorganisms are destroyed (from the CDC's Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008).

To achieve sterilization, steam under pressure must be provided at a particular temperature for an appropriate length of time. The four parameters that determine the effectiveness of this method are:

1. Steam. The ideal steam is dry saturated steam with a dryness fraction (the proportion, by weight, of dry steam in a mixture of steam and water) of $\geq$ 97% with a minimal amount of

non-condensable gases and minimal contamination by chemicals or particulates. The steam must also contact all areas of the item(s) being sterilized, so that autoclave bags and other containers should be left partially open to permit the entry of steam and ensure adequate contact.

2. Pressure. Pressure serves as a means to obtain the high temperatures necessary for sterilization.
3. Temperature. An autoclave uses steam under pressure (approximately 15 psi) to achieve a chamber temperature of either of the two common temperatures of 121^0C (250^0F) or 132^0C (270^0F). These temperatures must be maintained for a minimal time in order to destroy all microorganisms.
4. Time. A minimum cycle time of 30 minutes at a chamber temperature of 121^0C is commonly recommended for sterilization. However, the total time required to achieve sterilization depends on several other factors, including the nature of the material (solid or liquid), the volume of liquid, the shape and size of the containers, the density or viscosity of the material, and the microbial load. Recommendations for cycle times, considering several of these factors, are given in the accompanying Autoclave Safety and Operating Instructions (**Attachment B**).

Autoclave Maintenance and Monitoring

Autoclaves must be regularly maintained, repaired, and monitored for successful sterilization through the use of biological indicators (the use of a material carrying microorganisms such as Geobacillus or Bacillus species known to be highly resistant to the sterilization process).

VII. OSHA BLOODBORNE PATHOGENS STANDARD

The Bloodborne Pathogens Standard, incorporating the Needlestick Safety and Prevention Act of 2000, is designed to limit occupational exposure to blood and other potentially infectious materials (OPIM) that could result in the transmission of Human Immunodeficiency Virus 1 (HIV 1), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and other bloodborne pathogens.

The occupational routes of exposure to bloodborne pathogens, particularly in a laboratory setting, are through accidental puncture with contaminated needles or other sharps, contact of abraded/damaged skin with blood or other infectious materials, through the mucous membranes of the eyes, nose, and mouth, and via potential respirable aerosols (through vortex mixing, centrifugation, and sonication).

In addition to human blood, other potentially infectious materials (OPIM) include:

- Human blood components
- Products made from human blood
- Semen
- Vaginal secretions
- Cerebrospinal fluid
- Synovial fluid
- Pericardial fluid
- Peritoneal fluid
- Amniotic fluid
- Saliva in dental procedures
- Any body fluid visibly contaminated with blood, and all body fluids where it is difficult or impossible to differentiate between the fluids
- Any unfixed tissue or organ from a human (living or deceased)
- HIV 1, HBV, and HCV-containing cell cultures, organ cultures, and culture medium or other derived liquids
- Blood, organs, or other tissues from experimental animals infected with HIV 1, HBV, HCV, or other infectious agents
- Any other material of human or non-human primate (NHP) origin that may be potentially infectious not listed above

Bloodborne pathogens are defined as pathogenic microorganisms that are present in human blood and in other infected tissues or materials, and can cause disease in humans. These pathogens include, but are not limited to:

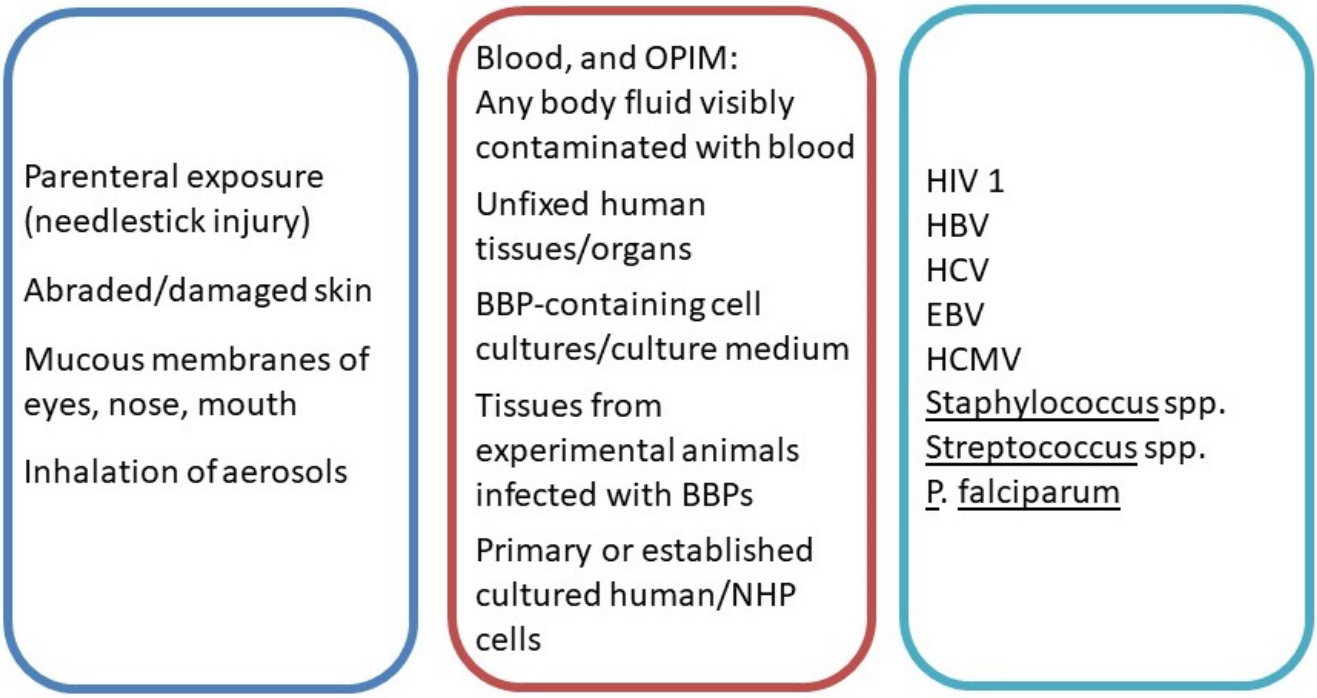
- HIV 1 (Human Immunodeficiency Virus 1)
- HBV (Hepatitis B Virus)

- HCV (Hepatitis C Virus)
- EBV (Epstein Barr Virus)
- HPV (Human papillomavirus)
- HTLV viruses (Human T-lymphotropic Viruses)
- HCMV (Human cytomegalovirus)
- Ebola virus
- Treponema pallidum
- Staphylococcus spp.
- Streptococcus spp.
- Mycobacterium tuberculosis
- Plasmodium falciparum, P. vivax, P. ovale, P. malariae

Additional information on these and other bloodborne pathogens can be obtained from the NIH Guidelines (April 2019), the BMBL, 6th Edition, and particularly the Pathogen Safety Data Sheets from the Public Health Agency of Canada, Laboratory Biosafety and Biosecurity. **Figure 3** offers a condensed summary incorporating occupational exposures, other potentially Infectious materials (OPIM), and several bloodborne pathogens.

Figure 3. OSHA Bloodborne Pathogens Standard

Standard limits **occupational exposure** to **blood** and **other potentially infectious materials (OPIM)** that could result in transmission of **BBPs**.



Universal precautions, as presented in the OSHA Standard, is an approach to infection control in which all human blood, certain body fluids, fresh tissues, and cells, as well as the OPIM mentioned above, are handled as if they are known to be infected with HIV 1, HBV, and/or other blood-borne pathogens. Included in this approach are specific engineering controls/safety equipment and safe work practices which further serve to minimize exposure to materials contaminated with infectious agents.

Engineering Controls (with the addition of safety equipment) includes:

- Hand washing areas, readily accessible to all personnel
- Personal protective equipment
- Sharps containers
- Emergency eye wash stations
- Autoclaves
- Biological safety cabinets
- Storage containers - leak-proof, puncture-resistant, which can be securely closed
- Transport containers - leak-proof, puncture-resistant, which can be securely closed
- The replacement of glass Pasteur pipets with plastic pipettes whenever possible

Work Practices (which also incorporate good microbiological practices) entails that:

- Personnel will wash their hands after the removal of gloves and other personal protective equipment and when work is begun or completed.
- Contaminated needles are not to be recapped, bent, broken, or removed from syringes, or otherwise manipulated by hand before disposal. Contaminated sharps are to be placed in sharps containers immediately after use.
- Broken glassware that may be contaminated is only picked up using mechanical means, such as brush and dustpan or by forceps.
- Good microbiological practices are to be employed whenever human blood or other potentially infectious materials are being handled.

The Bloodborne Pathogens Standard and Human and Non-Human Primate Tissues, Cells, And Cell Lines

Cells derived directly from human or non-human primate tissues, and even established cell lines from those sources, may under certain circumstances be considered other potentially infectious materials (OPIM; see **Figure 3**). In response to a query from the American Biological Safety Association regarding the applicability of the Standard to established human cell lines, OSHA prepared an Interpretation where established human cell lines (whether transformed through spontaneous mutations or through immortalizing viruses) characterized as free from contamination by HIV 1, HBV, and other recognized bloodborne pathogens are not considered to be OPIM and are therefore not covered by the Standard. In contrast, established human or other animal cell lines which are known to be or likely infected or contaminated with agents classified as bloodborne pathogens are covered by the regulations. The characterization of these cell lines would be accomplished by screening for viruses identified as bloodborne pathogens, as well as any bacterial and fungal contaminants and human mycoplasmas as necessary.

In addition, several other factors intrinsic to a cell can influence the contamination status: the species of origin, the cell or tissue type, and the culture status (these factors are listed in **Table 4**). The importance of the species of origin, the human or non-human primate nature of a cell, comes from an increased risk of infection from a pathogen as the genetic relationship of a cell approaches that of a human and the pathogen's species-specific barrier correspondingly decreases.

The cell or tissue type is also another factor to consider. A ranking according to cell type is presented in **Table 4**; blood would present the greatest risk of being contaminated with pathogens, followed by neural cells, endothelium, and then fibroblasts. This ranking is also identical to that provided in Appendix H of the BMBL 6th Edition where the tumorigenic potential by cell type is considered; the potential decreases again starting from hematopoietic cells through to epithelial cells and fibroblasts.

Finally, the culture status of the cell line is an important indicator of the presence or absence of bloodborne pathogens. Primary cell cultures are often derived directly from unfixed human or non-human primate tissues and must be produced frequently due to the limited lifespan of those cultures. If the tissues are contaminated by bloodborne pathogens (and thus considered OPIM), then a primary cell line derived from those tissues will very likely contain the same pathogens. This primary culture is in sharp contrast to the well characterized cell line described in the above Interpretation of the OSHA Standard. The characterized cell line has been extensively passaged and examined by genomic sequencing, diagnostic PCR, and cytometric analyses, and therefore presents a very low risk of pathogen transmission.

A risk assessment of cells and cell lines which incorporates the species of origin, cell or tissue type, and culture type is illustrated in **Table 4** below. The pathogens that contaminate a human cell line are most likely to be transmitted through an accidental puncture from contaminated needles, contact

through abraded skin, or through the mucous membranes of the eyes, nose, and mouth.

Table 4. Risk Assessment of Tissues and Cultured Cells

Increasing Risk ↑	Species of origin	Cell/tissue type	Culture type
	Human cells	Hematopoietic cells	Primary cell line
	Non-human primate cells	Neural cells	Continuous cell strain (finite cell divisions)
	Mammalian cells (e.g., murine)	Endothelium	Continuous cell line (unlimited cell divisions)
	Avian and invertebrate cells	Epithelial cell, fibroblast	Well-characterized cell line

(Modified from https://www.biosafety.be/content/contained-use-animal-cell-cultures-risk-assessment-and-biosafety-recommendations and Pauwels, K. et al., 2007; OSHA Standard 29 CFR 1910.1030, Standard Interpretation: https://www.osha.gov/laws-regs/standardinterpretations/1994-06-21)

The immortalization of human or non-human primate cells in the creation of cell lines must also be included among the risks of working with these lines. Whether through use of a virus or through transfection with recombinant DNA, the nature of the transgene introduced into the cell and the vector used should be considered and assessed. Any genetically modified cell line should be evaluated for newly acquired properties as compared to the original cell phenotype, as well as its permissiveness toward viral replication. Furthermore, human cell lines that have been demonstrated to be tumorigenic are indicated as potential hazards by the BMBL, 6th Edition, from the view of self-inoculation and subsequent tumor development.

In addition, the intentional or inadvertent infection of cells by pathogens can confer new properties to the infected cells and alter the risk assessment that was originally based on the uninfected cell line. The deliberate infection of cells with pathogens should consider the inherent properties of the infecting agent in determining the potential hazards presented by the infected cells. In the case of an adventitious infectious agent, the risk assessment becomes more complex as the agent may be unknown, and a combination of detection or identification methods may need to be employed.

As recommended in Appendix H of the BMBL 6th Edition, human and non-human primate cells should, at a minimum, be treated as potentially infectious and be handled using BSL 2 containment and procedures. All work should be performed in a biosafety cabinet as best practice, PPE (lab coats, gloves, and eye protection as required) should be worn, and all cell culture waste must be

decontaminated by autoclaving or disinfection prior to disposal. All laboratory members working with human and non-human primate tissues, cells, and cell lines should adhere to the policies and guidelines set forth in OSHA's Bloodborne Pathogen Standard.

Biohazard Signs and Labels

Biohazard signs must be posted at entrances to any Biosafety Level 2 (or higher) laboratory. The sign must include the following information:

- The universal biosafety symbol:

- Name of the infectious agent(s)
- The biosafety level of the agents
- Requirements for entering the facility, along with any required PPE
- Name and telephone numbers of the laboratory director and other responsible individuals

The following items must also be labelled with the universal biohazard symbol:

- Refrigerators, freezers, incubators, or other equipment containing blood or other potentially infectious materials
- Sharps disposal containers
- Contaminated equipment
- Containers used to store or transport blood or other infectious material

Individual cages used to house animals injected with BSL 1 or BSL 2 viral vectors should be labelled with a cage card displaying the biohazard symbol, the IACUC protocol number and the PI's name, the particular virus used, the date of injection, and the name and telephone number of a contact person for the laboratory.

VIII. BIOLOGICAL WASTE DISPOSAL

The definition of biological waste and the types of waste, along with an outline of the procedures by which laboratory personnel may properly handle and dispose of waste generated in the course of working with BSL 1 and BSL 2 agents, is provided below. The procedures by which biological waste, including recombinant nucleic acids and microorganisms, is handled and finally disposed of can include inactivation on-site or the packaging and storage of materials for eventual transfer and sterilization off-site.

Medical or biological waste, as defined in 105 Code of Massachusetts Regulations (CMR) 480.000: Massachusetts Department of Public Health, is waste which:

- May cause, or significantly contribute to, an increase in mortality or an increase in serious irreversible or reversible illness; or
- Pose a substantial present potential hazard to human health or the environment when improperly treated, stored, disposed of, or otherwise managed.

The types of medical or biological waste covered by the regulation may be briefly described as:

1. Blood and blood products - bulk human blood and blood products, body fluids contaminated with visible blood.
2. Pathology waste - human anatomical parts, organs, tissues, and body fluids removed and discarded during surgery, autopsy, or other medical or diagnostic procedures; specimens of body fluids and their containers.
3. Cultures and stocks of infectious agents and associated biologicals – all discarded cultures and stocks of infectious agents and their associated biologicals, including culture dishes and devices used to transfer, inoculate, and mix cultures, that are generated in:
   Laboratories involved in basic and applied research,
   Laboratories intended for educational instruction,
   Clinical laboratories.
4. Contaminated animal waste – contaminated carcasses, body parts, body fluids, blood, bedding from animals known to be:
   Infected with the zoonotic diseases (e.g. anthrax, pathogenic strains of avian influenza, bovine spongiform encephalopathy, tuberculosis, and tularemia),
   Infected with diseases designated by the State Epidemiologist as presenting a risk to human health,
   Inoculated with infectious agents for purposes including, but not limited to, the production of biologicals or pharmaceutical testing.

5. Sharps – discarded medical articles that may cause punctures or cuts, including but not limited to all needles, syringes, lancets, Pasteur pipettes, broken glassware, scalpelblades, suture needles, and disposable razors used in a medical procedure.
6. Biotechnology by-product effluents – any discarded preparations, liquids, culture, contaminated solutions made from microorganisms and their products including genetically altered microorganisms.

Waste Treatment Procedures

The various forms of waste that may be generated have corresponding appropriate handling and treatment methods, and the list below covers whether the waste is inactivated on-site, or removed by a third-party vendor for subsequent off-site inactivation/incineration.

Solid waste:

The waste must be packaged in an autoclave bag, and should not be packed more than three-quarters full. The bag must be open at the top (a minimum of two inches in diameter) to permit the entry of steam and to prevent bursting, yet be secure to prevent the loss of materials during handling and transport. Furthermore, the bag must be impervious to moisture and resist tearing, and should be stickered with autoclave tape. The waste should be transported in an autoclavable secondary container, preferably a polypropylene tray. The waste may then be sterilized using the appropriate autoclave cycle and time, as per **Attachment B** of this Handbook. After the material has been autoclaved, the autoclave tape should indicate that the sterilization cycle has been completed, and the bag may then be placed in an opaque or black trash bag in an adjacent trash container and disposed of as general solid waste.

Liquid waste:

Liquid waste in a flask should be combined with sufficient bleach to reach a final concentration of 10%. The solution should be left to incubate for 15 minutes in order to inactivate any hazardous agents. The liquid should then be discarded in the sink, and the sink rinsed extensively with water.

Liquids such as spent cell culture or bacterial media, for example, remaining after subculture or harvesting of the cells may be treated with bleach in the manner described above. However, liquid media which has come into contact with phenol or chloroform, through extractive processes, or other hazardous chemicals as defined in the Massachusetts Department of Environmental Protection 310 CMR 30.000, should be treated as chemical waste (as per the University Chemical Hygiene Plan http://www.brandeis.edu/ehs/labs/) and subsequently removed by Triumvirate Environmental (contact phone number 781-736-2561).

Sharps:

As previously covered under the **Safety Equipment** section of the Handbook and reiterated here, sharps should be handled so that:

1. Needles must not be recapped, bent, sheared, broken, or removed from disposable syringes before disposal.
2. Used disposable needles and syringes must be carefully placed in a puncture-resistant container used for sharps disposal. Do not fill the container greater than 75% full. For disposal call Ext. 6-2561; the contractor, Triumvirate Environmental, is on-site on Mondays and Thursdays.

3. Non-disposable sharps (surgical scissors, fine forceps, reusable needles) must be placed in a puncture-resistant container for transport to a processing area for cleaning and sterilization.

4. Contaminated glassware (having been in contact with human cells, infectious microorganism, viruses, etc., and whether broken or not) must be placed in a sharps box. Uncontaminated broken glassware must not be handled directly but must be removed using a brush and dustpan, or forceps, and placed in a broken glass box that has a plastic liner and is labelled "Broken Glass" (labels are available from EH&S). This box may be closed when less than 25 lbs. in weight, sealed with tape, and left for the custodial staff to remove.

   Plasticware should be substituted for glassware whenever possible.

Sharps containers that are full are to be collected by Triumvirate Environmental by contacting the firm at 781-736-2561 to arrange for pick-up.

Cells and cell lines

Cells and cell lines may be lysed by the addition of 10% bleach to the culture plate; the liquid is subsequently siphoned off into the liquid waste flask and the remaining culture plate is placed into the solid waste container. Any plastic pipettes/pipette tips having come in contact with the cells or cell supernatant is disposed of as solid waste and autoclaved. Disposable glassware such as Pasteur pipettes which may be used to remove cell culture media, and any sharps such as syringes with needles, are placed in a sharps container.

Bacterial cultures and associated culture ware:

Bacterial cultures are inactivated through the addition of bleach to a final concentration of 10% of the culture fluid, left for 15 minutes to inactivate all cells, and then poured into the sink. Bacterial agar plates are disposed of in a plastic-lined container provided by Triumvirate Environmental; once the container is nearly full, the waste disposal firm can be contacted to collect the material. All pipette tips and plasticware that have come into contact with the bacterial culture are placed in an autoclave bag and subsequently sterilized.

Disposal of contaminated animal tissues or waste:

Animal remains or tissues should be enclosed in a leak-proof bag or container and placed in a freezer located within the Foster Animal Facility. For animals that have been injected with BSL 1 or BSL 2 viral vectors, the tissues should be placed within a leak-proof wrapper and deposited in the section of the freezer specifically designated for virus research. In those instances where bedding has come in contact with animals immediately following the injection of recombinant viral vectors, the bedding must be disposed of into a biohazard bag followed by the wipe-down of the animal cage with the disinfectant Clidox (Foster Animal Facility).

IX. SELECT AGENTS AND TOXINS

The Federal Select Agent Program, a collaborative effort between the Centers for Disease Control and Prevention (CDC) and the Animal and Plant Health Inspection Service of the United States Department of Agriculture (APHIS-USDA), oversees the possession, use and transfer of biological select agents and toxins (http://www.selectagents.gov/). These agents have the potential to pose a severe threat to public, animal, or plant health or to animal or plant products. The Program is charged with:

- Developing, implementing, and enforcing the Select Agent Regulations.
- Maintaining a national database.
- Inspecting entities that possess, use, or transfer select agents.
- Ensuring that all individuals who work with these agents undergo a security risk assessment performed by the Federal Bureau of Investigation/Criminal Justice Information Service.
- Providing guidance to regulated entities on achieving compliance to the regulations through the development of guidance documents, and conducting workshops and webinars.
- Investigating any incidents in which non-compliance may have occurred.

A list of the select agents and toxins, extracted from the website, is given below:

HHS AND USDA SELECT AGENTS AND TOXINS LIST (7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73)	
HHS SELECT AGENTS AND TOXINS	**OVERLAP SELECT AGENTS AND TOXINS**
Abrin[6] Botulinum neurotoxins*,[6] *Bacillus cereus* Biovar *anthracis** Botulinum neurotoxin producing species of *Clostridium** Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)[6] *Coxiella burnetii* Crimean-Congo haemorrhagic fever virus Diacetoxyscirpenol[6] Eastern Equine Encephalitis virus[4,5] Ebola virus* *Francisella tularensis** Lassa fever virus Lujo virus Marburg virus* Monkeypox virus[4] 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) Ricin[6] *Rickettsia prowazekii* SARS-associated coronavirus (SARS-CoV)[5] 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 Saxitoxin[6]	*Bacillus anthracis** *Bacillus anthracis* Pasteur strain *Brucella abortus* *Brucella melitensis* *Brucella suis* *Burkholderia mallei** *Burkholderia pseudomallei** Hendra virus Nipah virus Rift Valley fever virus Venezuelan equine encephalitis virus[4.5]

HHS AND USDA SELECT AGENTS AND TOXINS LIST
(7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73)

HHS SELECT AGENTS AND TOXINS

South American Haemorrhagic Fever viruses:

- Chapare
- Guanarito
- Junin
- Machupo
- Sabia

Staphylococcal enterotoxins (subtypes A, B, C, D, E)[6]

Tetrodotoxin[6]
T-2 Toxin[6]

Tick-borne encephalitis complex (flavi) viruses:

- Far Eastern subtype[5]
- Siberian subtype[5]

Kyasanur Forest disease virus[5]

Omsk hemorrhagic fever virus[5]
Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus[4]
Classical swine fever virus[5]
Foot-and-mouth disease virus*,[5]
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum[4]
Mycoplasma mycoides[4]
Newcastle disease virus[3,4]
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus[5]

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

*Coniothyrium glycines (*formerly *Phoma glycinicola* and *Pyrenochaeta glycines)*
Peronosclerospora philippinensis (Peronosclerospora sacchari)
Ralstonia solanacearum[7]
Rathayibacter toxicus
Sclerophthora rayssiae[7]
Synchytrium endbioticum
Xanthomonas oryzae

*Denotes Tier 1 Agent – a subset of select agents or toxins that present the greatest risk of deliberate misuse with the most significant potential for mass casualties or deleterious effects on the economy, critical infrastructure, or public confidence. PIs who possess, use, or transfer Tier 1 select agents must adhere to additional regulations detailed within the Select Agent Regulations.

[2] C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins a-MI and a-GI (shown above) as well as a-GIA, Ac1.1a, a-CnIA, a-CnIB; X1 = any amino acid(s) or Des-X; X2 = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X3 = Arginine or Lysine; X4 = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X5 = Tyrosine, Phenylalanine, or Tryptophan; X6 = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X7 = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

[3] A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (Gallus gallus) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

[4] Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus, west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies Mycoplasma capricolum except subspecies capripneumoniae (contagious caprine pleuropneumonia), all subspecies Mycoplasma mycoides except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia), and any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, provided that the individual or entity can verify that the agent is within the exclusion category.

[5] For determining the regulatory status of nucleic acids that are capable of producing infectious forms of select agent viruses, please reference guidance here.

[6] For determining the regulatory status of Recombinant and/or Synthetic nucleic acids that encode for the toxic form(s) of any select toxins if the nucleic acids (i) can be expressed in vivo or in vitro, or (ii) are in a vector or recombinant host genome and can be expressed in vivo or in vitro; please reference guidance here.

[7] Select agents or toxins that meet any of the following criteria are excluded from the requirements of this part: Any subspecies of *Ralstonia solanacearum* except race 3, biovar 2 and all subspecies of *Sclerophthora rayssiae* except var. *zeae,* provided that the individual or entity can identify that the agent is within the exclusion category.

(Modified from the Federal Select Agent Program, https://www.selectagents.gov/sat/list.htm; 4/26/210)

Any individual or university/institution/entity that intends to possess, use, or transfer any select agent or toxin, including receipt of select agents or toxins from outside the United States, must register with either the:

- Department of Health and Human Services (HHS)/Centers for Disease Control and Prevention (CDC)/Division of Select Agents and Toxins (DSAT, or
- U.S. Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)/ Division of Agricultural Select Agents and Toxins (DASAT).

Both of these Departments, and the indicated centers and offices therein, are collectively known as the Federal Select Agent Program.

A complete *APHIS/CDC Form 1-Application for Registration, Use and Transfer of Select Agents and Toxins* must be submitted to the Federal Select Agent Program for review. The application may be submitted to either DSAT or DASAT but not to both, as the DSAT and DASAT work together to provide a single point of contact, with either serving as a "lead agency". This lead agency is responsible for coordinating all activities and communications with respect to the application for registration, including any communications with the non-lead agency. A designated DSAT or DASAT representative is assigned to the individual/entity once the Federal Select Agent Program has completed an initial review of the application. Time to approval of the application will depend on the scope and complexity of the registration; an inspection of the entity/institution may be required prior to approval.

Additional information on the Select Agents Regulations and Policies may be found at the website (http://www.selectagents.gov/regulations.html), as well a list of frequently asked questions, classified by topic, to assist in determining the appropriateness of registering with either the CDC or the APHIS/USDA for select agent possession or use and in applying for a registration (http://www.selectagents.gov/faq.html).

Permissible Toxin Amounts

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

HHS Toxins	Amount
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Excluded Strains of HHS and USDA Select Agents and Toxins

The Federal select agent regulations establish a process by which an attenuated strain of a select biological agent or toxin that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the regulations.

Based upon consultation with subject matter experts and a review of relevant published studies and information provided by institutions requesting exclusions, the Federal Select Agent Program determined that the following attenuated strains or less toxic select toxins are not subject to the requirements of the select agent regulations.

Any excluded select agent strain or modified toxin will be subject to the regulations if there is a reintroduction of factor(s) associated with virulence, toxic activity, or other manipulations that modify the attenuation such that virulence or toxic activity is restored or enhanced.

Nontoxic HHS Toxins

- Botulinum neurotoxins
- Conotoxins
- Staphylococcal Enterotoxins (SE)

Excluded Toxins Modified to be Less Potent or Toxic

- Tetrodotoxin

Excluded Attenuated Strains of HHS Select Agents

- Botulinum neurotoxin producing species of *Clostridium*
- *Coxiella burnetii*
- Eastern Equine Encephalitis virus
- Ebola virus
- *Francisella tularensis*
- Junin virus
- Lassa fever virus
- Monkeypox virus
- SARS-Coronavirus
- *Yersinia pestis*

Excluded Attenuated Strains of Overlap Select Agents

- *Bacillus anthracis*
- *Brucella abortis*
- *Brucella melitensis*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Rift Valley Fever Virus
- Venezuelan Equine Encephalitis virus

Attenuated Strains of USDA-only Select Agents Excluded

- African swine fever viruses
- Avian influenza virus (low pathogenic)
- Avian influenza virus (highly pathogenic)
- Foot-and-mouth-disease virus

Restricted Experiments

In addition to the select agents and toxins, the use of these materials in "restricted experiments" are regulated, such that an individual or entity may not conduct or possess products resulting from the following experiments unless approved by and conducted in accordance with the conditions prescribed by the HHS Secretary or APHIS Administrator:

1. Experiments that involve the deliberate transfer of, or selection for, a drug (or chemical) resistance trait to select agents that are not known to acquire the trait naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture.

2. Experiments involving the deliberate formation of synthetic or recombinant DNA containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD50 less than 100 ng/kg body weight. Note: Currently, only nucleic acids containing genes for the biosynthesis of Botulinum neurotoxin meet the definition of the regulation [42 CFR § 73.13(b)(2)(b)(2)].

3. Experiments that involve the creation of 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, or vice versa.

The HHS Secretary or APHIS Administrator may revoke approval to conduct any of the experiments listed above, or revoke or suspend a certificate of registration, if the individual or entity fails to comply with the requirements of this part of the regulations. To apply for approval to conduct any of the experiments above, an individual or entity must submit a written request and supporting scientific information. A written decision granting or denying the request will then be issued.

X. DUAL USE RESEARCH OF CONCERN

Dual use research (DUR) is research conducted for legitimate purposes that generates knowledge, information, technologies and/or products that can be used for both benevolent and harmful purposes. A good portion of life science research can be considered to encompass dual use, with the resulting information and/or techniques conceivably being used toward good or malevolent ends. Dual use research of concern (DURC) is a subset of dual use research, and is defined as "life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security" (http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research- concern).

The process of identifying DURC-related risks and the management of those risks is one driven by the Principal Investigator, relying on the identification of research that utilizes certain agents and/or experimental goals as defined by the DURC Policy. If a project involves one of 15 specific pathogens or toxins and any of seven different categories of experiments, the PI can alert an institutional review entity (IRE) so that the project can be evaluated according to the Policy. The role of the IRE can be fulfilled by any one of several different options: as a committee directly established to deal with DURC reviews, through the current IBC, or by an externally administered committee. As a result, a process which complements established federal reviews, encouraging both investigators and institutions to consider dual use issues and establish a culture of responsibility, would be achieved. The relationship between select agents and dual use research is also a complementary one, in that select agents represent very specific microorganisms and toxins while dual use research can encompass a large area of biological research, with dual use research of concern covering that portion of research where the derived knowledge, information, products, or technologies may be intentionally misused.

The DURC Policy lists a series of subsequent steps that an institution is to follow once a researcher has alerted the IRE that a project may qualify as DURC. The multistep process may be briefly described as follows.

After notification, the IRE is to determine if the U.S. funding agency has already identified the project as DURC. If not, the IRE verifies the PI's conclusion that the project involves one of the 15 defined pathogens and one of the seven kinds of experimental effects governed by the Policy. If the funding agency has already identified the research as DURC, then the institution must implement an approved risk mitigation plan and continue to monitor the research.

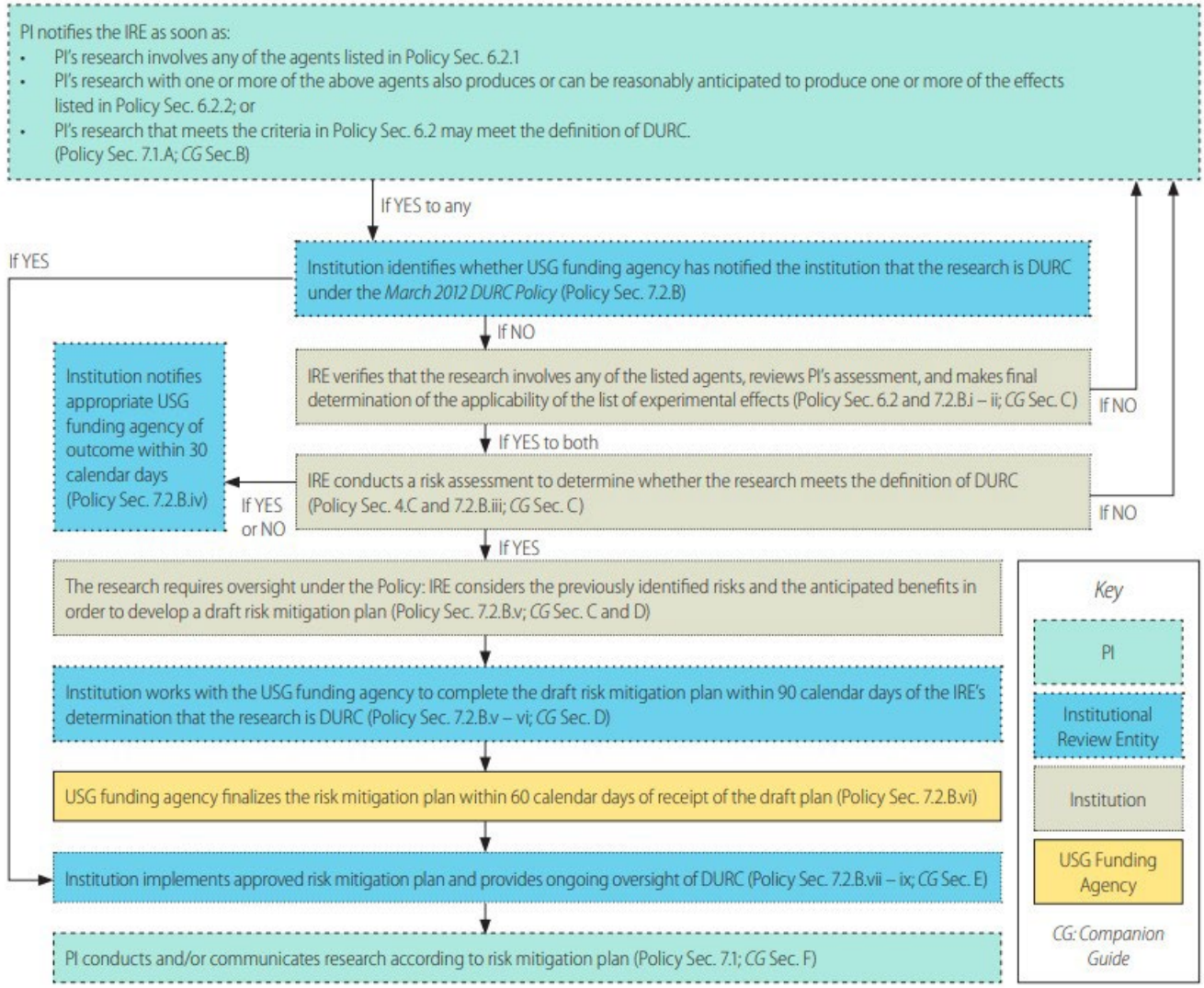
If it is the IRE that confirms the PI's conclusion, the committee then conducts a risk assessment to determine if the project meets the DURC definition. If the assessment is in the affirmative, the panel is expected to weigh the risks and anticipated benefits and, working with the government funding agency, write a draft risk mitigation plan within 90 days.

The funding agency then finalizes the risk mitigation plan within 60 days of receiving the institution's draft. Finally, the institution is required to implement the plan and provide ongoing oversight of the research.

A flow chart to help PIs and institutions with the process for review and oversight of research falling within DURC is found within a downloadable *Dual Use Research of Concern – A Companion Guide* (available at http://www.phe.gov/s3/dualuse/Documents/durc-companion-guide.pdf.) and is presented below in **Figure 4**. The *Companion Guide* also provides additional information on the identification, assessment, management, and responsible communication of research findings of DURC.

The DURC Policy applies to institutions and their investigators that receive federal funding for life sciences research, as well as those conducting research, funded by any source, involving any of the 15 agents and toxins (in any quantity) listed in the Policy.

Figure 4. Process for Institutional Review of Life Sciences Research within the Scope of the DURC Policy



(Extracted from the *Dual Use Research of Concern – A Companion Guide*, p. 3)

The 15 specific pathogens and toxins listed within the DURC Policy are:

- Avian influenza virus (highly pathogenic)
- Bacillus anthracis
- Botulinum neurotoxin
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Ebola virus
- Foot-and-mouth disease virus
- *Francisella tularensis*

- Marburg virus
- Reconstructed 1918 influenza virus
- Rinderpest virus
- Toxin-producing strains of *Clostridium botulinum*
- Variola major virus
- Variola minor virus
- *Yersinia pestis*

Note: the 15 biological agents listed above are also subject to select agent regulations.

The DURC definition further specifies seven categories of experiments, where the research aims to or is expected to:

1. Enhance the harmful consequences of the agent or toxin;
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical and or agricultural justification;
3. Confer to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alter the host range or tropism of the agent or toxin;
6. Enhance the susceptibility of a host population to the agent or toxin; or
7. Generate or reconstitute an eradicated or extinct agent or toxin listed in the 15 mentioned above.

Frequently asked questions about the U.S. Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern

A list of questions regarding the DURC Policy, to help a PI become familiar with the scope of the Policy and the attendant Institutional and Investigator responsibilities, has been presented as a PDF from the NIH (http://www.phe.gov/s3/dualuse/Documents/durc-faqs.pdf) . For detailed information, the U.S. Policy on DURC may be obtained at http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern.

ATTACHMENT A

INSTRUCTIONS FOR BIOSAFETY CABINET (BSC) USE:

Prior to use:

1. Wash your hands with soap and water and put on the appropriate personal protective equipment (PPE; at a minimum gloves, with a disposable gown/lab coat worn during virus preparation).
2. Raise the sash (glass panel) to the proper height (8 inches).
3. Turn on the blower and light.
4. Wipe down the work surface, grills, and interior surface of the sash with the appropriate disinfectant.
5. Load the cabinet with the necessary materials and reagents; make sure to disinfect the outer surface of bottles and pipettes before putting them in the BSC.
6. Include a container with disinfectant for discarded pipette tips and microcentrifuge tubes.
7. Let the air purge for 10 minutes.
8. Have an appropriate waste container close to the BSC for used pipettes, plates, and gloves.

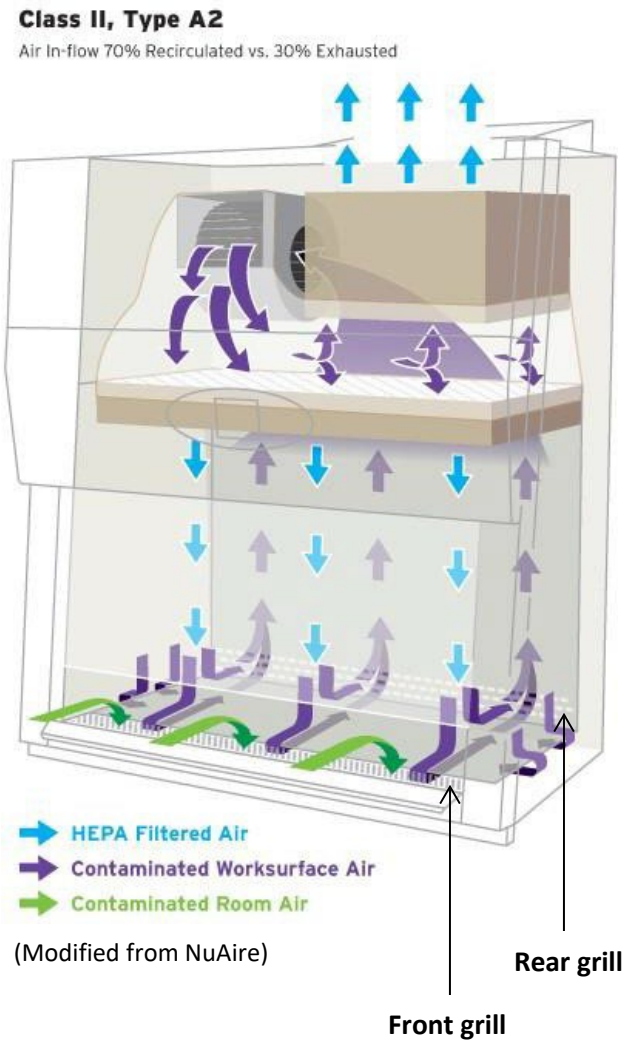
While working in the BSC:

1. Do not block the front and rear air intake grills.
2. To avoid disrupting the protective airflow pattern, avoid rapid movements in and out of the cabinet.
3. Establish working areas; always work from the "clean" to the "dirty" area.
4. Clean spills as soon as they occur and replace worn/torn gloves.
5. Protect the vacuum line of the liquid waste trap; use an in-line hydrophobic filter to protect the system from contamination.

After use:

1. Purge the air inside the BSC for several minutes.
2. Disinfect the materials/supplies that have been inside the cabinet before removing them.
3. Remove and discard waste materials.
4. Wipe down the BSC.
5. Turn off the blower and light and close the sash.
6. Remove PPE and wash hands.

Biosafety cabinet design and air circulation:



(Modified from NuAire)

BSCs are to be annually tested and certified.

ATTACHMENT B

BASIC AUTOCLAVE OPERATING INSTRUCTIONS

The following instructions do not replace the manufacturer's operating instructions or hands-on training. Familiarize yourself with the particular autoclave for the first time, as each autoclave presents unique operating features; contact an experienced user in the laboratory for instruction on safe operation.

1. Check the drain screen to ensure that it is not plugged or obstructed.
2. Place the items to be autoclaved in the chamber; load/stack to allow efficient steam circulation. Use a secondary container such as an autoclavable polypropylene tray to hold items. Autoclave clean items and waste separately. Use heat sensitive indicator tape to identify processed items.
3. **Close the autoclave door snugly**; the autoclave senses that the door has been closed, not necessarily sealed correctly.
4. Using the autoclave keypad/dials/buttons, select:
   A. The type of cycle: dry or liquid.
   B. The sterilization time: the sterilization time will vary according to the contents and how the load is arranged. The time should be measured after the temperature reaches 121°C (250°F) at a pressure of 15 pounds per square inch. A tray with large bags or containers will require a longer time to reach 121°C, and the time should be set accordingly.
   C. The sterilization temperature remains set at 121°C (250°F).
   D. Caution: never autoclave bleach, or flammable or radioactive materials.
5. Run the autoclave cycle. **Check that the autoclave reaches the correct temperature and pressure before leaving.** If steam is exiting from around the door, either tighten the door or abort the cycle.
6. At the completion of sterilization, the dry cycle will rapidly exhaust to zero pressure, while the liquid cycle will slowly release pressure so that liquids do not boil over (this may take up to 45 minutes).
7. **Open the door only after chamber pressure has returned to zero, slowly and slightly to allow steam to escape. Check gauges to confirm chamber pressure is at zero.** Leave the door open for several minutes to allow pressure to equalize and for materials to cool. Use appropriate protective equipment before unloading the chamber; wear heat resistant gloves and preferably closed-toe shoes.
8. Allow liquids to stand for 10 minutes before transfer.
9. Check the autoclave tape for a color change, as well as the circular chart recorder to confirm that the appropriate time and temperature were attained. The tape only indicates exposure to steam and heat, not sterilization. If the conditions were not met, the load should be re-autoclaved in another machine.
10. Dispose of autoclaved waste in an opaque trash bag set within a large trash container. The trash bag will be sealed prior to disposal in the regular waste.

Guide to Autoclave Cycles and Times

	Biological Waste (Dry Cycle)	Liquids (Liquid Cycle)	Dry Items (Dry Cycle)	Glassware (Dry Cycle)
Preparation	Open bag ~2 inches, place in tray. Mark with autoclave tape.	Loosen caps. Fill containers to no more than 50% capacity. Place flasks in tray. Mark with autoclave tape.	**Instruments:** Clean, dry, lying flat on tray. Mark with autoclave tape.	**Clean:** washed, rinsed, and wrapped. Mark with autoclave tape.
Placement in Autoclave	In the center.	In the center; upright in tray.	**Instruments:** Flat on tray.	**Clean:** upright or on side.
Temperature	121°C	121°C	121°C	121°C
Treatment Time	60-120 min. depending on load size and packing density	<100 ml - 25 min. <500 ml – 35 min. 500 ml to 1L – 45 min. 2L – 60 min.	30-60 min.	30-60 min.
Exhaust Cycle	Fast exhaust	Slow exhaust	Fast exhaust/dry	Fast exhaust/dry
Notes	Avoid puncturing bags. Dispose in plastic-lined trash containers.	Hot bottles may explode; cool before removing from autoclave.	Package instruments to allow steam circulation.	Glassware with cracks or deep scratches may break.