UMassAmherst | Environmental Health & Safety

Biosafety Manual

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Contents

Introduction

This Biosafety Manual was prepared by the Environmental Health & Safety (EH&S) Department after careful review of pertinent federal and state government regulatory documents, along with reference guidelines from the Centers for Disease Control, the National Institutes of Health, and the World Health Organization.

This manual will:

- Discuss components of working in a research laboratory;
- Address the most commonly asked questions from faculty, staff and students on general biosafety, pathogens, and recombinant DNA (rDNA) or synthetic nucleic acid (sNA) issues;
- Provide information about training, safe work practices, safety equipment and personal protective equipment; and
- Provide guidance for investigators who need to submit a protocol for review by the Institutional Biosafety Committee.

Due to the ever-changing regulatory environment, updates to this manual will be made as needed; these changes will also be made on the EH&S Biosafety website.

Record of Changes

Date	Version	Description of Change	Approved By

Recommended changes to this document should be forwarded to the EH&S Biosafety Program at 413-545-2682 or by e-mailing jladuc@ehs.umass.edu.

Chapter 1: Biosafety at a Glance

A snapshot of minimum requirements to work with rDNA/Infectious Agents, and associated lab requirements.

Approvals (Chapter 4)

Panel	Oversight	Website
IBC	rDNA/ Infectious Agents BSL-2, 3 (in vitro, in vivo)	http://www.umass.edu/research/biological-safety-and-ibc
IACUC	Use of animals	http://www.umass.edu/research/compliance/animal-subjects
IRB	Clinical trials, use of certain human specimens	http://www.umass.edu/research/compliance/human-subjects-irb

Trainings (Chapter 6)

Content	Where
Biosafety for Laboratory Staff	OWL (classroom and online)
Bloodborne Pathogens	OWL
BSL-3 Training	In-person
Laboratory Safety	OWL (classroom and online)
Fire Safety	OWL (classroom)
Autoclave Safety	OWL (online)
Animal User Training	Research Admin & Compliance (classroom)
Working with the IACUC	CITI (Research Admin & Compliance) online

Occupational Health (Chapter 7)

What	Contact Information	Who
Surveillance Program	Occupational Health (UHS) 413-545-5000	Works with/exposure to hazards

Lab Animals	Occupational Health (UHS) 413-545-5000	Works with/exposed to animals

Safety (Chapter 9)

Tools	When	How
Biosafety Cabinets	Annual certification/repairs/moves	EH&S 413-545-2682
PPE	Per risk assessment	EH&S 413-545-2682

Chapter 2: Roles and Responsibilities

The University is committed to fostering a culture of safety for all faculty, staff, and students. Such a culture enables the fulfillment of the University's mission to create an environment where teaching, learning, service, outreach, discovery, and creativity can flourish. Each of us plays a role in creating this environment. Each individual in the laboratory must be constantly aware of their surroundings and engaging in ongoing risk assessments and experiment planning. While it is important to abide by policies and rules meant to mitigate known risks, it is of even greater importance to be able to assess risks on a situational basis. Safety considerations should be part of all experimental designs from the very start, and should be continually addressed throughout the research process. Principal Investigators (PIs) and Responsible Individuals for laboratories should engage in discussion of safety considerations with their laboratory personnel and encourage discussion amongst their personnel of particular topics when appropriate. Department chairs should provide necessary and appropriate support for PIs to encourage attention to important safety considerations particular to their departments. When each individual is committed to safety, a strong sense of community identity invested in safety will emerge and benefit all.

The Chancellor has delegated to each dean, director, department head/chair, and supervisor the responsibility for safety performance within their respective units. Environmental Health and Safety (EH&S) and the campus safety committees help ensure that campus policies, as well as state and federal mandates, are followed.

Administrative Responsibilities

Environmental, Health and Safety (EH&S) is available to provide additional oversight, training, consultation, and technical assistance. Specific responsibilities are outlined below.

Responsibilities of Department Heads / Chairs

- Implement University safety and health policies
- Designate a Department Laboratory Safety Coordinator
- Ensure compliance with existing health and safety policies
- Review and grant approval for laboratory operations that involve particularly hazardous materials or processes
- Review and approve of all procedures and experimental apparatus used in the handling of extremely toxic gases, and gases with a high potential for explosion.
- Ensure hazardous materials are properly disposed of when researchers leave the university

Principal Investigator (PI)

A Principal Investigator is the primary individual responsible for the preparation, conduct, and the administration of a research grant, cooperative agreement, training or public service project, contract, or other sponsored project in compliance with applicable laws and

regulations and institutional policy governing the conduct of sponsored research. For purposes stated in this document, the PI is also responsible for ensuring that university health and safety policies are adhered to in their laboratory.

Faculty

Faculty are responsible for ensuring that university health and safety policies are adhered to in their laboratory.

Supervisors

Supervisors are responsible for ensuring that all university health and safety policies are adhered to in their areas.

Responsible Individuals

All PIs, faculty, supervisors, lab directors, or other individuals who are responsible for ensuring that all university health and safety policies are adhered to in the areas they are responsible for.

Responsibilities are to:

- Maintain accurate and up-to-date written research protocols that emphasize safety measures to be taken and personal protective equipment to be worn
- Conduct inspections in his/her labs to ensure compliance with existing policies.
- Inform all laboratory staff and students under his/her supervision of the potential hazards associated with laboratory operations, signs and symptoms of exposure to hazardous materials in use, and procedures for dealing with incidents and/or injuries
- Assure that staff, students, and employees under his/her supervision are trained as required by federal and commonwealth regulations, best management practices, and university policy to provide a safe working environment that is generally recognized to be free of hazards and ensure that all lab personnel are complying with the aforementioned requirements.
- Implement corrective actions for remediation of safety and regulatory issues including when provisions of this document are not being met within the lab
- Ensure the safety of all visitors to the laboratories.
- Supervise the laboratory to ensure that safe practices, personal protective equipment, and engineering controls are employed and used properly
- Instruct laboratory staff on the location and use of safety equipment in the facility
- Ensure that laboratory workers complete and submit appropriate forms for obtaining authorization for working with biohazardous materials or processes
- Ensure that laboratory workers understand how to work safely with biological materials
- Report incidents and any other safety problems to the Biosafety Officer and EH&S

Instructors and Teaching Directors

The role of instructors and teaching directors is to ensure that University health and safety policies are adhered to by staff, students, and visitors in teaching laboratories.

Responsibilities are to:

- Maintain accurate and up-to-date written laboratory protocols that emphasize safety measures to be taken and personal protective equipment to be worn
- Inform all laboratory staff and students under his/her supervision of the potential hazards associated with laboratory operations and procedures for dealing with incidents and/or injuries
- Supervise the laboratory to ensure that safe practices, personal protective equipment, and engineering controls are employed
- Report incidents and any other safety problems to the Department Laboratory Safety Coordinator and EH&S

Responsibilities of Department Laboratory Safety Coordinator

- Attend the Department Laboratory Safety Coordinator meetings
- Communicate to faculty and staff members University safety and health policies
- Report safety related incidents and potential safety problems that come to their attention to EH&S
- Specific duties may be assigned by the department

Responsibilities of Employees, Visiting Scholars, Students

This section applies to all graduate and undergraduate students in research laboratories.

- Follow all safety and health procedures in the laboratory as specified in the Laboratory Health and Safety Manual / Chemical Hygiene Plan and by the faculty supervisor or responsible individual
- Attend required health and safety training sessions. Students attending laboratory classes will receive safety training at the first class by the teaching assistant or instructor. All new employees and lab workers in research biological laboratories must attend a classroom based biosafety, lab safety, and fire safety training with EH&S prior to beginning work in the laboratory. The upcoming training schedule is available on the EH&S website. Annual refresher training via the Online Web-based Learning (OWL) system is also required for these Safety modules. The OWL system may be accessed at: https://owl.oit.umass.edu/, select the Environmental Health and Safety link under the trainings heading.
- Report incidents, unhealthy, and unsafe conditions to the faculty supervisor and EH&S
- Only conduct approved experiments for which there are written protocols in place
- Laboratory personnel are encouraged to notify the faculty supervisor of any pre-existing health conditions that could lead to a serious health situation in the laboratory

Responsibilities of Environmental Health and Safety

- Provide technical guidance on matters of laboratory safety
- Inspect laboratories to ensure compliance with safety and health guidelines and applicable regulations and to assist with remediation of safety issues
- Investigate incidents and recommend action to reduce the potential for recurrence
- Coordinate clean-up operations in the event of spills or other contamination
- Develop and conduct training programs in laboratory and biological safety, including the contents of this document
- Work with federal, state and local officials on matters of codes and enforcement
- Assist laboratory personnel with evaluating, preventing and controlling hazards
- Oversee the adoption and implementation of all University health and safety policies
- Maintain and update the University's Biological Safety Manual
- Post door cards for each laboratory that stores or uses biohazardous materials

Safety Committees

The following UMass/Amherst safety and ethics committees have been established in accordance with state and federal mandates and grant funding agency requirements: Radiation Use Committee (RUC), Institutional Biosafety Committee (IBC), Institutional Animal Care and Use Committee (IACUC), and the Institutional Review Board (IRB). An Institutional Chemical Safety Committee (ICSC) has also been established. The members of these committees are appointed by the Vice Chancellor for Research and Engagement. The responsibility of the committees shall be to establish safety, health, and ethical policies in accordance with federal, state, and local laws and regulations, institutional needs and best management practices, and to evaluate research being conducted on the UMass/Amherst campus for safety, health, and ethical considerations.

IACUC and IRB ensure that research and teaching at UMass Amherst that involves animal (IACUC) and/or human subjects (IRB) is conducted in compliance with state and federal laws pertaining to the health and welfare of the research subjects. These committees may refer research projects brought to them for review to the appropriate safety officer and/or safety committee for environmental health and safety review.

The IBC is the principal committee charged with advising on matters that relate to the safe use of biological materials in the laboratory environment. This includes reviewing and approving guidelines and standard operating procedures (SOPs) and practices for the use of biological materials. The IBC provides assurance that activities at the UMass/Amherst campus do not present unacceptable risks to the health and safety of faculty, staff, students, visitors, and the local community.

Risk Assessment for Research:

Evaluation and assessment of risk is a key part of designing and conducting an experimental protocol. Not only does a thorough risk assessment allow researchers to systematically identify and control hazards, but it also improves the quality of science through more thorough planning, a better understanding of the variables, and by sparking creative and innovative thinking. It allows one to implement tighter controls which reduces uncertainty and increases the safety and quality of your results/product. Failure to consider risk and hazards from the beginning of experimental design can produce delays, roadblocks, and frustration later in the process. There is a Risk Assessment template in Appendix H. The Risk Assessment process is broken down into four steps:

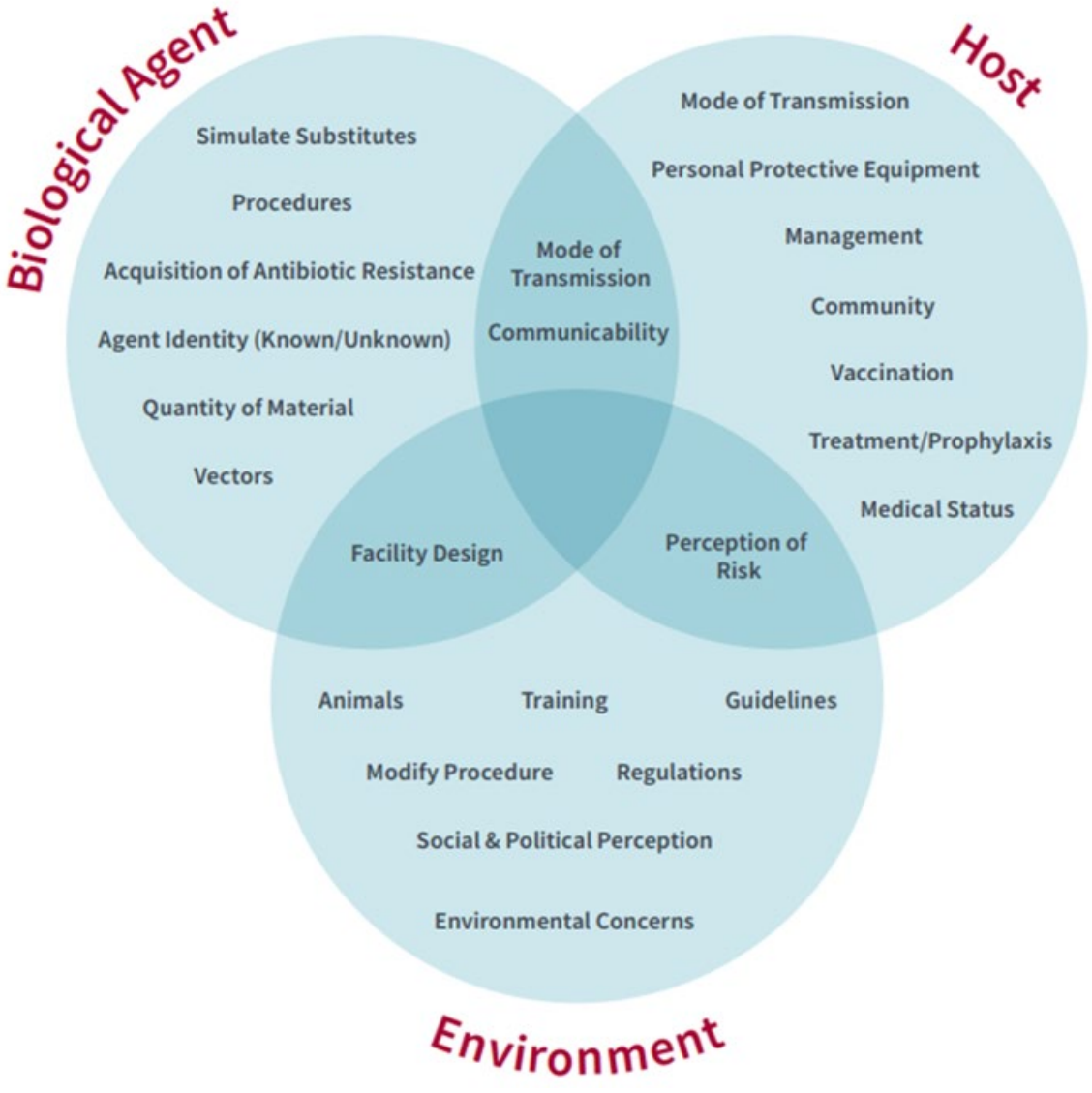
1. **Explore:**
   Determine the scope of your work, beginning with research objective. What question(s) are you trying to answer? Conduct a broad review of the literature. Speak with others who have done similar work. Are the risks different for different approaches?
2. **Plan:**
   Outline your procedure/tasks. This may include a deeper review into specific topics in the literature. Determine hazards associated with each step, and control measures for reducing risk. EH&S can help with more detailed guidance on how to handle certain hazards.
3. **Challenge:**
   What assumptions did you use? Question the importance of each step. Seek advice from others. Ask yourself "what could go wrong?" Have I missed anything? Consider all possible outcomes, how high is the risk?
4. **Assess:**
   Implement a model, prototype, or trial run. Can you perform a dry run to familiarize yourself with equipment and procedures? Can you test your experimental design at a smaller scale or with a less hazardous material? Determine if any design changes are needed. Run your experiment and monitor how your controls perform. Assess as you go and make changes as necessary.

Safety Culture and Biosafety

All of the above attributes form the basis for safe research but just like any science specialty, there are unique issues that must be considered when working with these materials, including:

- They can be alive and as such, can grow, replicate and sometimes, move.
- Their effect on the researcher can be influenced by the health of the researcher.
- They can spread through numerous mechanisms (droplet, aerosol, mucosal, oral, fecal, bloodborne).
- They can insert themselves into a genome and have long term effects.

The diagram below illustrates some of the many factors that must be taken into account when planning to work with biologicals and/or rDNA.



Chapter 3: Recombinant DNA & Synthetic Nucleic Acids: Regulations & Guidelines

NIH Guidelines

The use of recombinant DNA (rDNA) and synthetic nucleic acids (sNA) are regulated by the National Institutes of Health (NIH); the guidelines can be found in the publication NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). The guidelines are the official guide to all rDNA and sNA work done at UMass Amherst. It is important to realize that following these guidelines is the responsibility of all investigators at UMass Amherst and not solely that of investigators that are funded by NIH.

Exempt rDNA/sNA

The guidelines specify a number of different categories of rDNA/sNA molecules. One of the most important categories is the Exempt category. Experiments that qualify for this category do not need *approval* by the UMass Amherst Institutional Biosafety Committee. However, you must submit a protocol to the IBC to ensure that you have categorized your research correctly. To determine if your experiments are exempt, you can refer to Section III, Category F in the NIH Guidelines (online). A short reference guide is presented in Table 1 and 2.

Non-Exempt rDNA/sNA

If your experiment does not fall within the exempt categories (Table 2), you must obtain IBC approval.

Viral Vectors and Transgenes

All vectors are not the same. More importantly, the class of gene insert can change the Biosafety level of the construct. It is also important to realize that obtaining a cloning/expression vector from a commercial source does not mean it is automatically exempt or a BSL - 1. Table 3 lists many of the more common viral vectors in combination with different classes of inserts and their associated BSL level.

Human Gene Transfer

Protocols involving the use of rDNA/sNA for gene transfer into humans, whether done directly in the subject or in vitro and subsequently put into the subject, must be submitted to both the IBC and the UMass Amherst Institutional Review Board (IRB). Federal regulations require the IBC, upon receiving submission of a Human Gene Transfer protocol, to review the following aspects to determine if NIH Recombinant Advisory Committee (RAC) review is required:

- *The protocol uses a new vector, genetic material, or delivery methodology that represents a first-inhuman experience, thus presenting an unknown risk.*
- *The protocol relies on preclinical safety data that were obtained using a new preclinical model system of unknown and unconfirmed value.*

- *The proposed vector, gene construct, or method of delivery is associated with possible toxicities that are not widely known and that may render it difficult for oversight bodies to evaluate the protocol rigorously*.

Dependent upon the above findings the protocol will be either be submitted for RAC review or the IBC will state that RAC review is not required. For additional information concerning UMass Amherst's IRB panels, please access the panel's web site.

Table 1. Recombinant and Synthetic Nucleic Acid Molecules (NIH guidelines)

If your experiment is in an exempt category, IBC approval is not necessary but a protocol must be submitted for review. If your experiment does not fall within the exempt categories, you must have current IBC approval (also see Table 2).

Is your synthetic nucleic acid designed to: (1) neither replicate nor generate nucleic acids that can replicate in any living cell, and (2) not integrate into DNA, and (3) not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight?	Yes	Exempt (III-F-1)
Is your recombinant or synthetic nucleic acid molecule not in an organism, cell or virus and not been modified or manipulated to render it capable of penetrating cellular membranes?	Yes	Exempt (III-F-2)
Is your recombinant or synthetic nucleic acid molecule solely from a single source that exists contemporaneously in nature?	Yes	Exempt (III-F-3)
Is your recombinant or synthetic nucleic acid molecule solely from a prokaryotic host and propagated in the same host or transferred to another host by naturally occurring means?	Yes	Exempt (III-F-4)
Is your recombinant or synthetic nucleic acid molecule from a eukaryotic host and propagated in the same host?	Yes	Exempt (III-F-5)
Is your recombinant or synthetic nucleic acid molecule from species that naturally exchange DNA?	Yes	Exempt (III-F-6)
Does your genomic DNA contains a transposable element that does not contain any recombinant and/or synthetic nucleic acids?	Yes	Exempt (III-F-7)
Recombinant or synthetic nucleic acid molecule which does not present a significant risk to health or the environment, as determined by the NIH*	Yes	Exempt (III-F-8)

*The NIH has determined that rDNA/sNA from infectious agents of BL-2 (see Appendix A) or above is not exempt and must receive Biosafety approval. Additionally, certain cloning vectors, i.e. Adeno- or Sindbis-based vectors, or amphotropic MMLV based vectors, are some examples of rDNA that are non-exempt.

Table 2. Experiments requiring IBC approval

Approval required for experiments involving: (Specific NIH Guideline Section)	**Further Information and Examples:**
Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture. (III-A-1-a)	• Transferring a drug resistance trait that is used, had previously been used, may be used (including outside the U.S.), or that is related to other drugs that are used to treat or control disease agents. • Examples include transfer of: erythromycin resistance into *Borrelia burgdorferi*; pyrimethamine resistance into *Toxoplasma gondii*; chloramphenicol resistance into *Rickettsia conorii;* tetracycline resistance into *Porphyromonas gingivalis.*
Cloning of DNA, RNA or synthetic nucleic acid molecules encoding toxins lethal to vertebrates at an LD50 of <100 ng/kg body weight. (III-B-1)	• Cloning toxins (or using plasmids that express toxins with low LD50s). • Examples include: botulinum, tetrodotoxin, ricin, T-2, saxitoxin, abrin, tetanus, *Shigella dysenteriae* neurotoxin, pertussis, *Staph aureus* Beta, shigatoxin, and conotoxins.
Transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules into human research participants. (III-C-1)	• Use of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, that meet ANY of the following four criteria: o Contain >100nt, or o Possess biological o Have the potential to replicate in a cell, or o Can be translated or transcribed. • Examples include: use of a defective adenoviral vector to deliver the CFTR gene intranasally to patients with Cystic Fibrosis; introduction of an HSVTK transduced cell line into patients with epithelial ovarian carcinoma; introduction of a shRNA delivered in a plasmid, bacterial or viral vector.
Risk Group 2, Risk Group 3, Risk Group 4 or Restricted Agents used as Host-Vector Systems (III-D-1)	• The introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2, 3, 4, or Restricted Agents that meet ANY of the following criteria: o Have the potential to replicate in a cell, or o Possess biological properties that enable genome integration, or o Produce a toxin lethal to vertebrates at an LD50 of V 100 µg/kg body weight. • Examples include: Adenovirus, Herpes virus, Lentivirus, Amphotropic or VSV-g pseudotyped Murine Retrovirus, Human Retrovirus, Vaccinia virus Vesicular Stomatitis virus, and Adeno-Associated virus with helper virus.

DNA from Risk Group 2, Risk Group 3, Risk Group 4 or Restricted Agents cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. (III-D-2)	• Transfer of DNA from Risk Group 2, 3, 4, or Restricted Agents into nonpathogenic prokaryotes or lower eukaryotes. • Use of pathogens or defective pathogens as vectors. Examples include: Adenovirus, Herpes virus, Lentivirus, Amphotropic or VSV-g pseudotyped Murine Retrovirus, Human Retrovirus, Vaccinia virus Vesicular Stomatitis virus, and Adeno-Associated virus with helper virus.
Infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems. (III-D-3)	• rDNA experiments involving Risk Group 2, 3, or 4 pathogens. • rDNA experiments involving ≤ 2/3 of the genome from eukaryotic viruses in the presence of a helper virus. • Examples include: HIV, HTLV-I & II, West Nile Virus, and Lymphocytic Choriomeningitis Virus.
Whole animals, including transgenic animals. (III-D-4)	• Experiments utilizing any of the following that may lead to transmissible infection either directly or indirectly as a result of complementation or recombination in the animal: ○ > 2/3 of eukaryotic viral genome, or ○ Animals containing sequences from viral vectors, or ○ Stable integration of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germline. • Use of viable recombinant or synthetic nucleic acid molecule-modified Risk Group 2, 3, 4 or Restricted Agent microorganisms tested on whole animals.
Whole plants. (III-D-5)	• Experiments involving exotic infectious agents when recombinant or synthetic nucleic acid molecule techniques are associated with whole plants. • Experiments with plants involving cloned genomes of readily transmissible exotic infectious agents. • Experiments with plants involving readily transmissible exotic infectious agents (i.e. soybean rust fungus *Phakopsora pachyrhizi*, maize streak or other viruses) in the presence of their specific arthropod vectors. • Experiments involving plants or their associated organisms and the introduction of sequences encoding potent vertebrate toxins. • Experiments involving microbial pathogens of insects, arthropods or small animals associated with plants if the recombinant or synthetic nucleic acid molecule-modified organism can detrimentally impact the ecosystem.
Large-scale DNA work. (III-D-6)	• ≥ 10 liters of culture combined.

	• Examples include: Use of ≥10 L fermenter; growing up to five 2 L flasks of rDNA culture (i.e. E. coli K-12).
Influenza virus. (III-D-7)	• Experiments with Influenza virus shall be conducted at the BSL containment corresponding to the Risk Group of the virus that was the source of the majority of segments. • Experiments that alter antiviral susceptibility may increase containment level requirements. • Examples of BSL-3 influenza work: 1957-1968 Human H2N2, Highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/06-like H5 lineage (HPAI H5N1), 1918 H1N1.

a. *Refers to the parental or wild-type virus and some of the common deletions used in viral vectors. MMLV, Moloney murine leukemia virus; SIV, simian immunodeficiency virus.*
b. *Refers to ability of vector to infect cells from a range of species. Ecotropic generally means able to infect only cells of the species originally isolated from or identified in. Please note that the ecotropic host for HIV and HSV would be human cells, but the ecotropic host for MMLV would be murine cells. Amphotropic and VSV-G-pseudotyped virus host range includes human cells.*
c. *Shown are general categories of cellular genes and functions. Please note that there are differences in the containment level for the same class depending on whether the viral vector integrates into the recipient genome at a high rate. The general categories are as follows: S, structural proteins (actin, myosin, etc.); E, enzymatic proteins (serum proteases, transferases, oxidases, phosphatases, etc.); M, metabolic enzymes (amino acid metabolism, nucleotide synthesis, etc.); G, cell growth, housekeeping; CC, cell cycle, cell division; DR, DNA replication, chromosome segregation, mitosis and meiosis; MP, membrane proteins, ion channels, G-coupled protein receptors, transporters, etc.; T, tracking genes such as those for green fluorescent proteins and luciferases and photoreactive genes; TX, active subunit genes for toxins such as ricin, botulinum toxin and Shiga and Shiga-like toxins; R, regulatory genes for transcription and cell activators such as cytokines, lymphokines and tumor suppressors; Ov and Oc, oncogenes identified via transforming potential of viral and cellular analogs, or mutations in tumor suppressor genes resulting in a protein that inhibits/moderates the normal cellular wildtype proteins. This does not include SV40 T antigen. SV40 T-antigen-containing cells should not be considered more hazardous than the intact virus. SV40 is considered a risk level 1 agent (the lowest level) according to the NIH Guidelines. The prevalence of SV40 infection in the U.S. population due to contaminated polio vaccine does not seem to have caused a statistically significant increase in the rate of cancers. However, the data from the various studies on SV40 association with cancer are equivocal (Strickler et al. 1998; Butel and Lednicky, 1999; Dang-Tan et al., 2004).*
d. *This is a general assessment of containment levels for laboratory construction and use of these vectors for nonproduction quantities only based on the 4th edition of BMBL. This table cannot cover every potential use within a research or laboratory settings; as information is gained, risk assessments and containment levels may be changed. Local IBCs should use all available information and their best judgment to determine appropriate containment levels. BSL - 1* refers to the containment level based on parent virus risk group. However, most procedures involving the handling and manipulation of the viral vectors are done at BSL - 2 to protect cell cultures and viral stocks from contamination.*

e. *Certain specific strains of poxviruses, such as MVA, NYVAC, ALVAC and TROVAC, are considered low-risk agents and can be handled at BSL - 1 in certain cases. From Biological Safety Principles and Practices, 4th ed., pg. 524, D.O. Fleming and D.L. Hunt, Ed, ASM Press, 2006.*

Genome Editing and Gene Drives

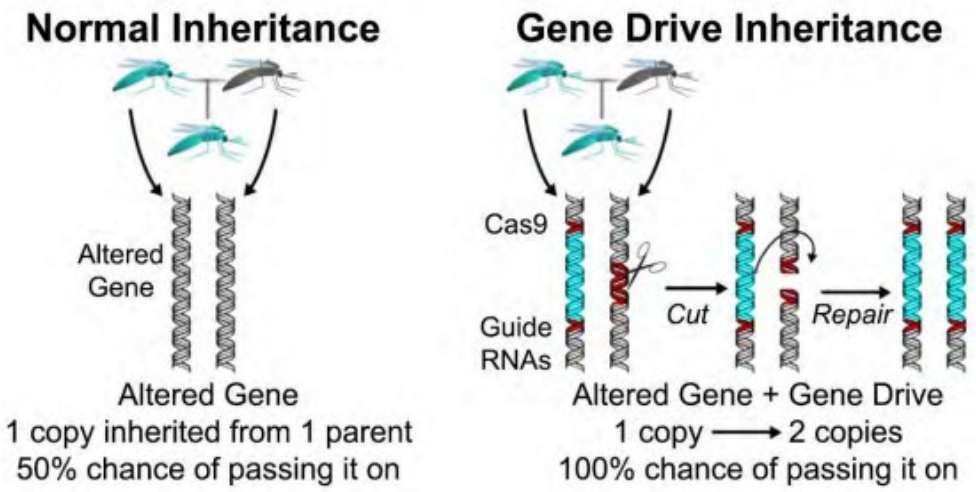


Figure 1. Genome Editing and Gene Drives
Image credit: Kevin Esvelt

Multiple technologies exist to create permanent genomic modifications in *in vitro* cell culture and *in vivo* animal research models (Figure 2). Methodologies include, but are not limited to, Transcription Activator-Like Effector Nucleases (TALENS), Zinc Finger Nuclease mediated DNA repair (ZNF), Meganucleases, and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) (Figure 1). These technologies can be used to create gene drives, a modification of an organism's genome resulting in a more efficient spread of a trait through the population as compared to Mendelian inheritance.
Per NIH regulation and as a requirement of UMass Amherst guidance, conducting genome-editing experiments on human embryos is prohibited.

Experiments that Require IBC Approval

Human Clinical Studies

- *Study protocols that include either direct gene modification or the administration of donor cells that have been genetically modified must be filed with both the IBC and Human Subjects Research (IRB).*

Basic Research Studies (In Vitro and In Vivo)

- ***Delivery via viral vectors:*** *Non-exempt viral vectors and Risk Group 1 viral vectors (e.g. AAV) with human target sequences. Genome target scans of the guide RNA (gRNA) sequence is highly recommended to identify the possibility of off target effects on the human genome. www.rgenome.net/cas-offinder*
- ***Usage of a gene drive (via viral or non-viral delivery methods) with invertebrate and vertebrate animals or on plants:*** *In addition to the description of the planned experiments and safety of the delivery mechanism, the IBC protocol must also address the following containment guidelines.*
    - *Molecular containment: Will the guide RNA and the nuclease be located in separate loci? Will a synthetic target sequence be used that is absent from the wild type target organism?*
    - *Ecological Containment: Will the experiments be performed outside the habitable range of the target organism?*
    - *Reproductive Containment: Will a laboratory isolate/organism be utilized that cannot reproduce with wild type organisms?*
    - *Barrier Containment: What physical and chemical barriers will be used to contain the target organisms and prevent their release into the environment?*
- ***Genome editing tools (delivered via viral or nonviral delivery methods) that:***
    - *Modify an infectious agent to increase host range, transmissibility, or pathogenicity of that particular agent.*
    - *Modify the host to increase its susceptibility to an infectious agent.*
    - *Express a toxin with a low LD50 (≤100 ng/kg) in the genome of both in vitro and in vivo research models.*

Exempt Experiments

Genome editing experiments that fall under the exempt category involve the use of nonviral transfection methods (e.g. electroporation, lipofection) to create genomic insertions, point mutations and deletions in somatic cells *in vitro* or *in vivo*. These insertions include rDNAs that express oncogenes or tumor suppressor genes. However, if the experiment involves the expression of a toxin with an LD50 toxin ≤100 ng/kg, the work becomes non-exempt and an IBC protocol submission and approval is required.

Transgenic Plants

Experiments to genetically engineer plants by rDNA/sNA methods may require registration with the IBC (BL2-P or higher, see OBA - NIH Guidelines and Appendix C for additional information). To prevent release of transgenic plant materials to the environment, the guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants. (Figure 3) Plant Biosafety levels are categorized into BL1-P to BL4-P (Table 4).

Transgenic Arthropods

Experiments to genetically engineer arthropods by rDNA/sNA methods may require registration with the IBC (ACL-2 or higher, see NIH Guidelines III-E-2-b-(5), Appendix L, and Appendix M). To prevent release of transgenic arthropod materials to the environment, the Arthropod Containment Guidelines (*BMBL 5th Edition*, Appendix E, pages 377 – 378), provide specific arthropod biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered arthropods. See Appendix D in this manual. Arthropod Biosafety Levels are categorized into ACL-1 to ACL-4. Version 3.2 of the Arthropod Containment Guidelines published by the *American Committee on Medical Entomology; American Society of Tropical Medicine and Hygiene* are here:

Arthropod Research Permits

Additional permits might be required from state and federal agencies before research with arthropods can be done.

United States Department of Agriculture Animal and Plant Health Inspection Service: Pests and Diseases search tool: https://www.aphis.usda.gov/aphis/resources/pests-diseases

- Permits should be applied for three months in advance of the beginning of the research.
- Permits are not transferrable to other individuals.
- Permits must be renewed 3 months prior to the expiration date listed on the permit.
- Read, and adhere to the **PERMIT CONDITIONS** that are listed on your permit. These conditions form the basis of the USDA inspector's checklist of compliance.

Table 3. Viral vectors and transgene containment.

Gene transfer vector	Host range	Insert or gene function	Laboratory containment level
MMLV based— *gag, pol*, **and** *env* **deleted**	Ecotropic Amphotropic, VSV-G pseudotyped	S, E, M, G, CC, T, MP, DR, R, TX, O_v, O_c S, E, M, MP, DR, T, G Ov, Oc, R, CC TX	BSL - 1* BSL - 2 BSL- 2+ BSL - 3
Herpesvirus based— nonlytic	Broad host range	S, E, M, MP, DR, T, G O_v, O_c, R, CC TX	BSL - 2 BSL-2+ BSL - 3
Lentivirus based— HIV, SIV, EIAV, FIV, etc.; *gag, pol, env, nef*, and *vpr* **deleted**	Ecotropic, amphotropic, VSV-G pseudotyped	S, E, M, MP, DR, T, G O_v, O_c, R, CC TX	BSL - 2 BSL-2+ BSL - 3

Adenovirus based— serotypes 2, 5 and 7; E1 and E3 or E4 deleted	Broad host range, infective for many cell types	S, E, M, T, MP, DR, R, G, CC O_v, O_c TX	BSL - 2 BSL-2+ BSL - 3
Alphavirus based— SFV, SIN	Broad host range	S, E, M, T, MP, DR, R, G, CC Ov, Oc TX	BSL - 2 BSL-2+ BSL - 3
Baculovirus based	Broad mammalian host cell range	S, E, M, T, MP, DR, R, G, CC O_v, O_c TX	BSL - 1* BSL-2 BSL - 2+/BSL - 3
AAV based— *rep, cap* **defective**	Broad host range; infective for many cell types, including neurons	S, E, M, T, MP, DR, G O_v, O_c, R, CC TX	BSL - 1* BSL-2 BSL - 2+/BSL - 3
Poxvirus based— canarypox, Vaccinia[e]	Broad host range	S, E, M, T, DR, MP, CC, R, G O_v, O_c TX	BSL - 2 BSL-2+ BSL - 3

Table 4. Plant biosafety levels.

From Practical Guide to Containment: Plant Biosafety in Research Greenhouses, Revised Edition, page 13, D. Adair and R. Irwin

Criteria	**Transgenic Plants**	**Transgenic Microbes**		**Transgenic Insects/ Animals/Assoc. Microbes**
		Exotic	**Non-Exotic**	
Not a noxious weed or cannot outcross with one	BL1-P			
Not easily disseminated			BL1-P	
No detriment to environment		BL2-P or BL1-P+	BL1-P	BL2-P or BL1-P+
Noxious weed or can interbreed with weeds	BL2-P or BL1-P+			
Contains complete genome of non-EIA*	BL2-P or BL1-P+			
Contains genome of EIA	BL3-P or BL2-P+			
Treated with an EIA	BL3-P or BL2-P+			
Detriment to environment		BL3-P-4**	BL2-P or BL1-P+	BL3-P or BL2-P+
Involves EIA with detriment to environment	BL3-P or BL2-P+			
May reconstitute genome of infectious agent in plants	BL3-P or BL2-P+			

Contains Vertebrate Toxin	BL3-P	BL3-P	BL3-P	

*EIA—Exotic Infectious Agent **BL4-P containment is recommended only for experiments with readily transmissible exotic infectious agents whether transgenic or not, such as air-borne fungi or viruses in the presence of their arthropod vectors that have the potential of being serious pathogens of major US crops.

Plant Research Permits

Additional permits might be required from state and federal agencies before research with plants can be done.

United States Department of Agriculture Animal and Plant Health Inspection Service: Pests and Diseases search tool: https://www.aphis.usda.gov/aphis/resources/pests-diseases

- Permits should be applied for three months in advance of the beginning of the research.
- Permits are not transferrable to other individuals.
- Permits must be renewed 3 months prior to the expiration date listed on the permit.
- Read, and adhere to the **PERMIT CONDITIONS** that are listed on your permit. These conditions form the basis of the USDA inspector's checklist of compliance.

Chapter 4: Infectious Agents: Regulations and Guidelines

Laboratories that work with infectious agents pose risks to people within the lab as well as nearby. Infections have been contracted in connection with laboratory work throughout the history of microbiology. Studies have illustrated that laboratory-acquired infections are not confined to any one kind of lab or group of people, and that the incidence of infection among untrained and ancillary workers is high, averaging approximately one-third of all acquired infections.

Tissue Culture, Human and Primate Tissue

The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency Virus (HIV), as well as agents that may be present in human tissues (e.g. *Mycobacterium tuberculosis, Streptococcus, Toxoplasma*, etc.) Non-human primate cells and tissues also present risks to laboratory workers (Herpes B virus), as do cells transformed with viral agents such as SV-40, EBV, or HBV, cells carrying viral genomic material and tumorigenic human cells. All are potential hazards due to the possibility of exposure.

Cultured cells which are known to contain or be contaminated with a biohazardous agent (i.e. bacteria or virus) are classified in the same BSL as the agent. Cell lines which do not contain known human or animal pathogens are designated BSL - 1. The following list contains human or primate cells that are to be handled using BSL - 2 practices and containment:

- Cells from blood, lymphoid cells, and neural tissue
- All primary cell lines
- Secondary (immortalized) cell lines
- Cell lines exposed to or transformed by a human or primate oncogenic virus
- Pathogen deliberately introduced or known endogenous contaminant
- Fresh or frozen tissue explants

Note that this list is not conclusive and individual cases will be determined as they occur.

Universal Precautions

Universal Precautions is the concept of treating all human/ primate blood and other body fluids, tissues and cells (including cell lines) as if they were known to be infectious for bloodborne pathogens.

All human blood, blood products, unfixed human tissue and certain body fluids shall be handled with Universal Precautions and BSL - 2 practices.

Table 1. Basis for the classification of biohazardous agents by biosafety level.

BSL 1	Agents that are not associated with disease in healthy adult humans
BSL 2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
BSL 3	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)
BSL 4	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)

Table 2. Summary of laboratory facilities for BSL 1 – 4.

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not associated with disease in healthy adults	Standard Microbiological Practices	As needed to allow for good microbiological practices	Open bench top Sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL - 1 practice plus: Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: Class I or II BSC or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPE: laboratory coats; gloves; face protection as needed	BSL - 1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL - 2 practices plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Primary barriers: Class I or II BSC or other physical containment devices used for all open manipulations of agents PPE: protective lab clothing, gloves, respiratory protection as needed	BSL - 2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life threatening disease, aerosol transmitted lab infections, or related agents with	BSL - 3 practices plus: Clothing change before entering Shower on exit All material	Primary barriers: All procedures conducted in a Class III BSC, or Class I or II BSC **in combination with** full-body, air-supplied, positive pressure personnel suit	BSL - 3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum and decon systems Other requirements

	unknown risk of transmission	decontaminated on exit from facility	outlined in the text of the BMBL

Work practice controls include frequent hand washing, no mouth pipetting, no food or drink in the lab and proper disposal of biohazardous/ medical waste, as well as the use of engineering controls and personal protective equipment (PPE). Engineering controls include biosafety cabinets, ventilation systems, closed top centrifuge rotors, etc.; these are the primary methods to control exposure. PPE such as gloves, lab coats, and eye protection or face shields must be selected and used as appropriate. All material should be treated as medical waste (see Chapter 9).

At all Biosafety Levels your last line of protection is the SINK. After finishing all procedures and cleanup, wash your hands with soap and water.

Areas subject to Universal Precautions must have appropriate signs posted on doors and equipment; these signs can be obtained from EH&S (413-545-2682).

Biological agents are classified by Risk Group (RG); RG 1 being the least pathogenic to RG 4 being the most. The RG, together with the work to be done (experiments) is assessed to determine the Biosafety Level (BSL).

Biosafety Level Classification

The categorizing of infectious agents into levels as described in Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF), is written and published by the Centers for Disease Control (CDC) and NIH. The BMBL describes combinations of microbiological practices, laboratory facilities, and safety equipment in combination with four biosafety levels for various agents infectious to humans. The descriptions of biosafety levels (BSL) 1 – 4 parallel those in the NIH Guidelines for research involving recombinant DNA. Biosafety levels are also described for infectious disease activities that involve laboratory animals or plants. It is important to note that the guidelines presented in the BMBL are considered minimal for containment, and will be customized as needed.

The BSL categories are divided up by risk of disease combined with availability of preventive and therapeutic treatments. The four groups are shown in Table 1. For the list of agents and their categories, see Appendix A or go to: https://my.absa.org/tiki-index.php?page=Riskgroups .

Laboratory Facility Requirements

Each BSL has its own corresponding requirements for the laboratory facilities. The physical requirements described in Table 2 will be used in conjunction with additional protective mechanisms (see Chapter 7) to achieve personnel and environmental safety.

Biosafety Level Work Practice Requirements

Each BSL is associated with work practices that address the potential risks. Along with practices for BSL-1, 2 and 3, is a list of practices labeled as BSL - 2+. This category is used for BSL - 2 agents that are worked with using BSL - 3 practices. (Table 3).

Biosafety Level 3 Laboratories

Biosafety Level 3 (BSL-3) laboratories involve research using agents that are associated with serious or lethal human disease for which preventative or therapeutic treatments may be available. These laboratories are designed to protect individuals and the public through the containment of the agents used by both engineering and administrative controls. Any waste generated from these facilities must be sterilized before disposal outside of the facility. Access to these laboratories are tightly controlled and involve a rigorous mentored training process designed by the laboratory to ensure that individuals are both capable and highly knowledgeable in all procedures involving the agent and facility in use. (Figure 1).

The primary hazards to personnel working with BSL - 3 agents involve autoinoculation, exposure to aerosols and ingestion. In order to prevent infection through these modes of transmission, containment devices such as a biosafety cabinet should be used along with procedures and practices that are thoroughly vetted before work begins. In addition to primary engineering and administrative controls, the facility itself must meet strict design guidelines to insure the environment and public are protected from any accidental release inside the facility.

Due to the intrinsic risk associated with work at BSL - 3, each researcher's knowledge and ability must be evaluated individually, with a gradient of experience and proficiency expected. All researchers must demonstrate competency with a minimum base skill set along with being secure in their potential to ask questions and express concerns. All labs should have an internal expert knowledge source to serve as a mentor to ensure specific skills are passed on.

To ensure the safety of all researchers and the environment, the IBC requires BSL - 3 researchers to demonstrate appropriate theoretical knowledge and practical skillsets in the following areas:

- Adhering to general lab safety procedures, including donning and doffing PPE.
- Setting up, cleaning out, and properly using the biosafety cabinets.
- Bringing materials into and out of the biosafety cabinet.
- Growing and manipulating cultures safely, with emphasis on the importance of avoiding aerosol generation during all operations.
- Performing the essential procedures required of most protocols, such as centrifugation, plating and incubating.
- Using the autoclave, disposing of waste.

- Emergency management and procedures.

Training should be thoroughly documented and consist of multiple sessions that culminate in a practical test to assess the skill of the researcher. These records should be kept in the lab and sent to the Biosafety group for evaluation. The results for these tests will also serve as the basis for access to the BSL-3 facility.
Formal training is strongly encouraged. There are a number of intensive BSL - 3 training courses offered across the United States; please contact the Biosafety group to discuss what is best suited for individual needs.

Contact Biosafety if you are considering doing research with a BSL-3 agent.

Select Agents and Toxins

Select Agents and Toxins are a collection of designated infectious agents and toxins that, by their nature, have the potential to pose a severe threat to public health and safety; this threat has resulted in the creation of a number of legislative acts.

Initiated with the Antiterrorism and Effective Death Penalty Act of 1996, and bolstered by the USA Patriot Act of 2001 and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, the Select Agents and Toxins program oversees the transfer, possession, and use of biological agents (viruses, bacteria) and toxins that have the potential to be a severe threat to public or environmental health. Possession of the specified agents or toxins without registration carries severe civil and criminal penalties.

Possession of Select Agents or Toxins over exempt amounts is not allowed at UMass Amherst at this time and would require prior approval from the IBC and registration with the FSAR Program. The application and further information may be found on the Federal Select Agents Registry (FSAR) website: https://www.selectagents.gov/SelectAgentsandToxins.html
UMass Amherst is currently not registered for possession of viable select agents. For use of any biological select agent, contact the Biosafety Program.

UMass Amherst Select Toxins Program

Possession of small quantities of select toxins may be exempt from registration with the NSAR (National Select Agents Registry) program. The UMass Amherst Select Toxins Program summarizes the University's requirements for possession of NSAR Select Toxins under the exempt quantities. For additional information, please contact the Biosafety Group.

Requirements for Research with Prions and Prion-Like Proteins

Per NIH guidelines (http://osp.od.nih.gov/officebiotechnology-activities/biosafety/nih-guidelines) and the UMass Amherst IBC (see Chapter 5, Charge to the IBC), the IBC requires

researchers to have an approved IBC protocol and follow specific guidelines for working with prions and prion-like proteins.

Prions and prion-like proteins are defined as proteins (human or animal) that fall into one of the below categories:

- Proteins that are highly associated with proteinopathies, including, but not limited to:
    - *Major prion protein/PrP/CD230 (Creutzfeldt-Jakob Disease [CJD], variant Creutzfeldt-Jakob Disease [vCJD], Kuru, fatal familial insomnia, bovine spongiform encephalopathy, Gerstmann Straussler-Scheinker syndrome)*
    - *Alpha-synuclein (Parkinson's disease)*
    - *Tau, beta-amyloid (Alzheimer's disease)*
    - *Tau, RNA-binding protein Fused in Sarcome (FUS) (Frontotemporal lobar dementias)*
    - *Polyglutamine-containing proteins (polyQ) (Huntington's disease)*
    - *Superoxide dismutase 1 (SOD1); transactivations response element (TAR) DNA-binding protein-43 (TDP43); RNA-binding protein Fused in Sarcoma (FUS); Ubiquilin (ALS/Lou Gehrig's disease)*
    - *Proteins that confer a disease state that is transmissible from cell to cell.*
- Proteins that have a fibrillar or aggregated form that has been shown to "seed" a pathology associated with a disease.

  Specific in vitro or in vivo work with such proteins is classified as BSL-2 or ABSL-2 and requires an IBC approved protocol. This includes, but is not limited to, the following types of work:
    - Synthesis, use or production of protein in high concentration
    - Generation or use of mutated proteins
    - Generation or use of fibrillar or misfolded forms of proteins

IBC protocols must include established prion disinfection/decontamination and destruction/ disposal protocols, or specific Standard Operating Procedures (SOPs). These SOPs must be provided for review by the IBC. If necessary, contact Biosafety for appropriate methods. Refer to the following references for established infection control guidelines for disinfection/decontamination:

- World Health Organization Infection Control Guidelines http://www.who.int/csr/resources/publications/ bse/WHO_CDS_CSR_APH_2000_3/en/
- Centers for Disease Control Prion Diseases https://www.cdc.gov/prions/index.html
- Biosafety in Microbiology and Biomedical Laboratories https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF Section VIII
- Biosafety Manual (see Chapter 11, Waste and Decontamination)

Dual Use Research of Concern (DURC)

A subset of research, as defined by the Federal government, that has the greatest potential for generating information that could be readily misused to threaten public health and national security has been termed “dual use research of concern” or DURC. (Figures 3 & 4)

The United States Government (USG) is presently limiting the scope of DURC policies to a subset of 15 biological agents and toxins that are considered Select Agents and are regulated by the US Department of Health and Human Services and the U.S. Department of Agriculture. Additionally there are 7 categories of experiments that come under DURC.

1. Avian influenza virus (highly pathogenic)
2. Bacillus anthracis
3. Botulinum neurotoxin
4. Burkholderia mallei
5. Burkholderia pseudomallei
6. Ebola virus
7. Foot-and-mouth disease virus
8. Francisella tularensis
9. Marburg virus
10. Reconstructed 1918 Influenza virus
11. Rinderpest virus
12. Toxin-producing strains of Clostridium botulinum
13. Variola major virus
14. Variola minor virus
15. Yersinia pestis

Categories of experiments

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

Work with these agents/toxins under these circumstances requires additional review (Figure 4). This review is multi-layered (Figure 3) and extensive. Contact Biosafety for further information or if you are planning any work that could be considered to fall under DURC.

Gain of Function Research

Certain gain-of-function (GOF) studies with the potential to enhance the pathogenicity or transmissibility of potential pandemic pathogens have raised biosafety and biosecurity concerns, including the potential dual use risks associated with the misuse of the information or products resulting from such research.

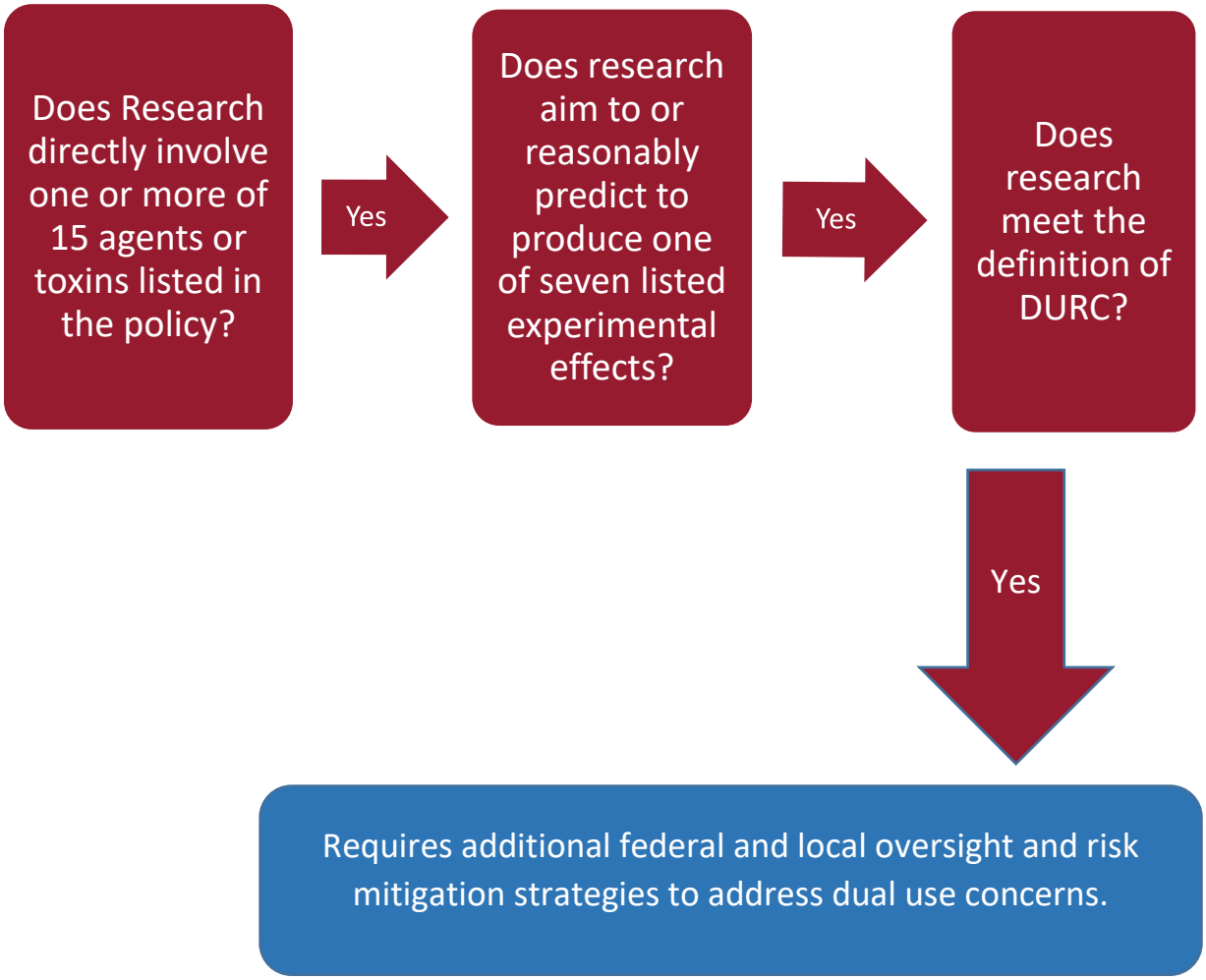
The U.S. Government undertook a deliberative process to re-evaluate the potential risks and benefits associated with certain GOF experiments. During this process the USG paused the release of federal funding for GOF studies anticipated to enhance the pathogenicity or transmissibility among mammals by respiratory droplets of influenza, MERS, or SARS viruses. The NSABB served as the official federal advisory body on the GOF issue and in May 2016, delivered recommendations to the USG on a conceptual approach for evaluating proposed GOF research.

Informed by this deliberative process, the USG released *Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight* in January 2017. (https://www.phe.gov/s3/dualuse/Documents/P3CO-FinalGuidanceStatement.pdf)

Figure 3. Overview of the Process for Institutional DURC Oversight:

PI	• PI identifies research that involves any of the 15 listed agents • Submits research protocol via eProtocol™ to the UMass Amherst Institutional Biosafety Committee
IBC	• Determines whether the research involves any of the 7 experimental effects • If so, conducts a risk assessment to determine whether the research is DURC; and • If so, weighs the risks and benefits and develops a draft risk mitigation plan
Government	• US Government funding agency finalizes and approves risk mitigation plan
IBC	• Institution implements approved risk mitigation plan and provides ongoing oversight
PI	• PI conducts and communicates research according to risk mitigation plan

Figure 4. Research Subject to DURC Policies:



Chapter 5: Institutional Biosafety Committee (IBC)

Institutional Biosafety Committee Compliance at UMass Amherst

The University of Massachusetts Amherst (the University) has an Institutional Biosafety Committee (IBC) in compliance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines, April 2016) and in accordance with Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, December 2009*.

The Institutional Biosafety Committee (IBC) is a University-wide review body that provides oversight over University operations and activities of a potentially biologically hazardous nature. The IBC reviews and approves research that involves potentially biohazardous materials (plant and animal pathogens, oncogenes, carcinogens, toxins and recombinant DNA), as required by University, State and Federal directives.

The IBC consists of no fewer than five members so selected that they collectively have experience and expertise in recombinant or synthetic nucleic acid molecules and technology. The committee shall also include a biosafety officer, one scientist with expertise in animal containment, and one scientist with plant, plant pathogen or plant containment principles. Two members shall be from the community and who represent the interest of the surrounding community with respect to health and protection of the environment. The committee has the capability to assess the safety of the proposed research and to identify any potential risk to public health or the environment.

The committee schedules monthly meetings and meets at least four times a year. Questions as to whether a material is a potential biohazard should be directed to the Biosafety team (EH&S) at 413-545-2682.

The IBC follows NIH Guidelines recommendations for reviewing projects that require constructing and handling: (1) rDNA and synthetic nucleic acid molecules, and (2) organisms and viruses containing rDNA and synthetic nucleic acid molecules. In addition, the IBC reviews activities involving use of Select Agents and Toxins and other biohazardous agents that must be handled at BSL-3. The IBC also reviews activities involving BSL-2 materials.

The IBC assists the EHS Biosafety Officer in formulating policies and procedures related to the use of biohazards. The IBC is also charged with reviewing the biological and medical waste management program annually and may advise the institution and the Principal Investigator (PI) concerning management of research that is classified as “dual use”.

IBC Oversight at a Glance

Biohazardous materials include any organism that can cause disease in humans, or cause significant environmental or agricultural impact, such as:

- Bacteria
- Viruses
- Parasites
- Prions and Prion-like proteins
- Fungi
- Human or primate tissues, fluids, cells, or cell cultures/lines that are known to or are likely to contain infectious organisms
- Human or animal tissues, fluids, cells, or cell cultures/ lines that have been exposed to infectious organisms
- Animals known to be reservoirs of zoonotic diseases

The IBC reviews the use of the recombinant and synthetic nucleic acid molecules. This includes:

- Recombinant and synthetic nucleic acid molecules
- Transgenic animals
- Transgenic plants
- Human gene transfer or studies using recombinant DNA

Examples of Recombinant DNA and Infectious Agents that must be approved by the IBC prior to use:

Biohazardous Agents

- Infectious/pathogenic agents classified in the following categories: Biosafety level 2, 3, and 4, or
- Other agents that have the potential for causing disease in healthy individuals, animals, or plants.

Recombinant DNA Molecules

- Molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell
- DNA molecules that result from the replication of those described above.

Synthetic Nucleic Acid Molecules

- Can replicate or generate nucleic acids that can replicate in a living cell
- Are designed to integrate into DNA
- Produce a toxin with a LD50100 nucleotides
- Can integrate into the genome

- Can replicate in a cell
- Can be transcribed or translated.

Gene Transfer

- Delivery of exogenous genetic material (DNA or RNA) to somatic cells for the purpose of modifying those cells.

Dual Use Research of Concern

- A subset of research, as defined by the Federal government, that has the greatest potential for generating information that could be readily misused to threaten public health and national security has been termed “dual use research of concern” or DURC.

Chapter 6: Training

The University offers numerous training courses and materials for employees of all levels and backgrounds. A basic list of required trainings for laboratory workers is available in OWL: https://ehs.umass.edu/owl-training

Biosafety Training for Laboratory Personnel

- Classroom training for Biosafety is required every five years. You may sign up for Biosafety Classroom training here: https://ehs.umass.edu/biological-safety-training-4
- Biosafety training is required to be completed online annually (in OWL)
- Autoclave Use and Procedures training is required for all individuals that use an autoclave

Bloodborne Pathogens Training

- Bloodborne Pathogens training is available online in OWL. The Bloodborne Pathogens Standard (29 CFR, Bloodborne Pathogens. - 1910.1030) applies to all staff that may have an occupational exposure to blood or other potentially infectious materials.

BSL-3 Biosafety Training

- In the BSL-3 laboratory environment, the type of experiments being conducted, nature of the material used, and the equipment used would determine the required types of training. Written documentation of BSL-3 training must be recorded and retained by the PI and Biosafety Office.
- Biosafety training for BSL-3 activities is provided by the Biosafety Officer, at least annually. Contact the Biosafety Officer to schedule these trainings.
- Task specific BSL-3 laboratory training is provided by the PI or a competent supervisor. Training competency checklists may be provided by Biosafety.
- If required, training and certification for shipping of dangerous biological materials and/or dry ice must be completed. Additional information on this is found in Chapter 10.

Biosafety Information Sessions by Request

- The Biosafety group offers a number of live and on demand Information Sessions to better suit your particular needs and that of your lab / facility; these can be done at a lab group meeting or any get together, for one lab or a department.

Emergency Lab Resources for Labs Working with Biological Materials

Emergency situations require prompt reaction and thoughtful use of available resources. While we can't prepare for every possible emergency, it is beneficial to discuss what resources are available and what we should be thinking about before and after an emergency.

Medical Emergencies:

When working with biological material, consider what information would be helpful to those assisting the injured. Be sure to discuss possible exposure to human pathogens or other potentially infectious materials.

- Call 911 or UMPD at: 413-545-3331
- If you can't call, then delegate task to the nearest individual.
- Where is the nearest AED?
- Where are your first aid supplies?
- Do you have a Safety Data Sheet in the case of an exposure?

Power Failure

Consider where your biological materials are stored and what equipment contains such materials.

- Are your freezers on backup power?
- How long will the backup power last?
- If not, where will you get enough dry ice to save your -80C freezers?
- Post sign and remind everyone to not open the freezer doors.
- Do you have trays available to catch the water leaking from the defrosting freezers?
- It's always good practice to keep your freezers free from ice buildup.
- Are your incubators on backup power?

Flood

You cannot "see" what biological material may be present in flood water. Do not step into water since hazards are unknown. Plan for the following situations:

- Do not wade into water where there is electrical equipment.
- Do you have research animals in the lab?
- Have flood waters reached research material?
- Is the power still on?
- Do you have a tarp available to cover valuable equipment?

Chapter 7: Medical Surveillance, Considerations and Advising

Work with biohazards, rDNA, animals, or materials falling under Universal Precautions guidelines may present medical concerns that trigger recommendations for medical surveillance.

Surveillance

Medical surveillance examinations may be required for researchers who work directly with biohazardous agents. Depending on the agent, the strain, and the work being done, such surveillance testing may be required annually or biannually, with the optimal protocols determined in consultation between the IBC, Biosafety, and University Health Center's Occupational Health Team.

- Upon completion of a medical examination, the participant will be notified by UHS of pertinent test results, with appropriate referrals made in the event of abnormal findings. The Occupational Health clinic will provide medical clearance as indicated to the requesting department.
- If there is a restriction indicated on the medical clearance that significantly limits an individual's ability to complete their job, then the supervisor shall notify the Biosafety Officer to discuss a remedial course of action.
- Medical records, including clearance paperwork, will be kept at the Occupational Health Clinic (UHS) for the duration of the individual's participation in the Medical Surveillance Program.

Occupational Health Services

UHS provides on-site services for UMass Amherst faculty and staff for work related:

- injuries
- illnesses
- medical surveillance
- immunizations

University Health Services (413) 577-5000
150 Infirmary Way
Amherst, MA 01003

Monday – Friday
8 a.m. – 8 p.m.

On-Site Services Provided

Medical Surveillance & Immunizations Medical surveillance is the process of evaluating workers' health as it relates to their potential occupational exposures to hazardous agents.

Medical surveillance, including:

- Tuberculosis screening
- Respirator use clearances
- Hearing tests
- Focused physical exams
- Urinalysis
- Blood tests

Immunizations:

- Hepatitis A
- Hepatitis B
- Varicella (chicken pox)
- MMR (Measles, Mumps, Rubella)
- Tetanus boosters
- Others as required by potential work exposures

Employee Work-related Injury & Illness Care

Initial and on-going care is provided.
Services include:

- First aid
    - Evaluation and management of work-related injuries/illnesses which may include: Medications
    - Exercise/stretching programs
    - Referral to physical therapy
    - Medical treatment (e.g., splints, crutches)
    - Work status reports
    - Diagnostic testing
- Examples of injuries and illnesses treated:
    - Cuts, abrasions
    - Sprains/strains (back, knee, wrist, etc.)
    - Repetitive stress injuries
    - Bloodborne pathogen exposures (e.g., needle sticks, blood splash)
    - Animal bites
    - Non-human primate exposures
    - Chemical exposures

Reporting Work-related Accidents, Incidents & Exposures of Employees

- Inform supervisor
- Report to UHS for treatment ASAP (within 2 hours)
- Complete a Lab Incident Report and submit to EH&S within 24 hours https://ehs.umass.edu/lab-incidents-and-lab-incident-report-form
- Complete a "Notice of Injury" form if you are an employee. This form is submitted to Human Resources to initiate Workman's Compensation Insurance benefits. Contact the Human Resource Worker's Compensation Specialist at: 413-545-6114, and ask for an NOI form. The NOI must be completed by the injured employee and signed by his/her supervisor. Return the NOI to HR within 48 hours of the incident.

Hepatitis B Vaccination Program

University employees with potential for any exposure to human or non-human primate blood, body fluids or any other substances covered by the Bloodborne Pathogens program (see Chapter 6) as a part of their work at UMass Amherst will be offered the Hepatitis B vaccine at no cost to the employee.

Hepatitis B vaccination can prevent hepatitis B and its consequences, including liver cancer and cirrhosis. Although Hepatitis B vaccine is made from parts of the hepatitis B virus, it cannot cause hepatitis B infection. The vaccine is usually given as 3 or 4 shots over a 6-month period.

Hepatitis B Vaccine Information Statement: https://www.cdc.gov/vaccines/hcp/vis/vis-statements/hep-b.pdf

Common agents for which a vaccine may be recommended

Hepatitis A virus
Hepatitis B virus
Influenza virus
Vaccinia virus
Poliovirus
Rabies virus
Salmonella typhi
Clostridium tetani (tetanus)
Varicella-Zoster virus
Measles-Mumps-Rubella (MMR)
Yellow-fever virus

Laboratory Animal Occupational Health

Primary goals of the program are to:

- Protect individuals from work-related risks associated with exposure to animals through a program of species-specific health information, education, and risk-based medical evaluation,
- Protect the health of research animals from certain transmissible diseases,
- Be pertinent to the species with which individuals are exposed and the work they perform

Allergies to Laboratory Animals

In the United States, an estimated 40 to 50 million people currently suffer from allergies. Hypersensitivity to household pets is a common problem in the population as a whole, and in the research setting, allergies to laboratory animals (ALA) can become a serious concern. Among those whose education or occupation requires significant exposure to lab animals, ALA affects approximately one in five people. Rats and rabbits are the most frequently implicated species, but mouse allergies are becoming more apparent, especially as the numbers of mice utilized increases and the research projects using them require more direct handling. Cat and dog allergies may also be occupational if a research project includes those species, and it is possible to develop allergic reactions to most other species, including hamsters and ferrets, after chronic exposure.

Although it is true that people with very limited contact develop fewer problems, studies have shown that the problem of ALA can be just as severe in those handling animals for scientific purposes (research staff) as it is in those responsible for their primary care (caretaking staff). A history of previous allergies (i.e. atopy) is not a guarantee that animal related problems will develop, but some studies have found a correlation between pre-existing atopy and ALA. The symptoms are generally evident six to 24 months after exposure but sometimes may take years to develop, and often worsen over time. Mild symptoms of ALA involve the eyes and nose (e.g. sneezing, runny nose, watery eyes) and/or the skin (itchy welts or rashes). Non-ALA allergies such as hay fever or an associated occupational allergy like latex hypersensitivity may cause ALA symptoms to worsen. These minor problems typically will not go away if the exposure to animal allergens does not change, and can ultimately progress to the most serious manifestation of ALA, which is animal-related asthma. Asthma is a serious, potentially debilitating problem, and it will predictably affect a percentage of workers who ignore the earlier symptoms of rhinitis or conjunctivitis (runny eyes and nose). Asthma is estimated to affect two percent of all people using animals during their first year of exposure, and an additional two percent per year thereafter.

ALA can have serious consequences for affected personnel, not just in terms of personal health, but in determining future career options as well. Studies have shown that about 50% of those with symptoms will eventually stop working with animals because of the discomfort involved

with ALA. Many of those people can change career tracks or be reassigned to non-animal duties within the same institution, but as many as 15% of affected workers will eventually quit their jobs because of ALA. Manifestations of asthma may not completely subside until six or more months after ending contact with animals.

The major allergens involving rodents are low-molecular weight proteins excreted in the urine, which adhere to skin and hair. They can also be found in soiled bedding, and may be distributed as airborne contamination in rooms where animals are housed or manipulated.

There are three general approaches to allergy treatment:

- avoiding the allergen through environmental control
- medication to relieve symptoms
- immunologic desensitization (allergy shots)

Medications can provide relief, but the following should be kept in mind:

- Although there are over-the-counter drugs which can give temporary symptomatic relief, it is best to seek the advice of a physician before self-prescribing. Drugs can mask the warning signs of developing asthma and may also cause drowsiness.
- If you use an antihistamine, take the drug prior to exposure for best results.
- Other types of anti-allergy drugs that do not induce drowsiness are now available by prescription, if necessary. Standard allergy shots (immuno-therapy) to reduce allergic sensitivity to cats and dogs have improved in recent years, and may be a good choice for some people.

Allergen avoidance is the only complete solution to ALA. If avoidance is impossible, it's critical that exposure is minimized as much as possible. Many people with ALA are able to continue working with animals by taking some simple precautions, such as the following:

- Wear personal protective equipment, including a tight-fitting mask, gloves, and a long-sleeved lab coat (or other dedicated uniform) at all times when working with animals. In some cases, a respirator or a filtered air-supplied face mask may be warranted.
- Take advantage of filter-topped caging (if available in the facility) to contain allergens when animals are transported or held. An understanding of the proper use of ventilated workstations will help minimize aerosol exposure when cages are opened.
- Avoid unnecessary exposure to irritants such as dust, tobacco smoke, and air pollution, since irritant chemicals can worsen airborne allergy symptoms.
- If you are experiencing symptoms of ALA, specialized medical professionals are available to help evaluate and treat your problems.

Procedures to minimize allergen contact (such as those listed above) should be followed by all exposed persons, even if ALA symptoms are not present, as these may prevent the

development of clinical signs. At the very least, following these procedures may greatly slow the progression of ALA. Current federal guidelines require that all personnel beginning to work with animals be given information regarding ALA and the precautions that should be taken. ALA should be treated like any other occupational health hazard and personnel should notify their supervisors or SUOHC of known or potential work-related allergies.

Field Work and Travel

Travel medicine related to field work or work-related travel, within the United States and abroad, is available via a consultation with University Health Services.

Biosafety is often consulted for help with risk assessments. Any student or staff doing work off UMass Amherst's campus or farther afield should make an appointment with UHS to discuss their work, any travel risks, and travel-related medicine, as well as how personnel health history might affect travel recommendations. This also includes any incidents, accidents or exposures that happen during travel. Personnel should seek appropriate medical attention as necessary during travel, and consult with UHS upon their return to campus. Travel registration with the International Programs Office (http://www.umass.edu/ipo/) is also highly recommended to aid with any medical or travel issues.

Risk considerations for travel and field work include what work is done and where (e.g., location and duration of stay or work), physical setting (e.g., terrain and proximity to water), the work being done (e.g., involvement of animals, chemicals, potential for dermal or respiratory hazards, etc.), access to engineering controls, and risks not specific to the work but specific to the location. These risks can include animals in the area, endemic infectious agents, political or local concerns, natural disaster potential, etc. Personnel engaging in travel and field work are advised to be aware of their surroundings, availability of local health care, and any specific issues or hazards associated with the work or area. Field work also presents some unique considerations for work in general, including exotic zoonotic concerns and importation of samples into the United States or Massachusetts. Specific permits may be required for materials from outside the state or country. Contact Biosafety for further information regarding the specific requirements for your particular work.

Special Cases

Special work circumstances may dictate the need for extra surveillance or medical clearance by UHS. Circumstances can include the species of animal being worked with (e.g., nonhuman primates, sheep, birds, wild animals), the agent being worked with (e.g., Mycobacterium tuberculosis), or the level at which the work is conducted (e.g., BSL--3). Certain health circumstances may also result in the need for consultation with UHS prior to work. If you have

questions or concerns about the nature of your work, please consult Biosafety by calling 413-545-2682. For health concerns, please call the University Health Services at 413-577-5000.

PPE – Personal Protective Equipment

A risk assessment of work or agents planned may dictate the need for specific PPE. One typical example of this is the need to wear an N95 respirator. In order to wear an N95 respirator, personnel must complete annual medical clearance and fit testing for enrollment in the N95 program. Examples of work requiring an N95 respirator or other PPE for respiratory protection may include work with BSL-3 aerosol-transmissible agents, work with BSL-2 aerosol-transmissible agents that cannot be done within a Biosafety Cabinet, field work that generates dust or debris potentially containing dried feces or urine, or a need for increased protection from allergens during animal work. Other examples of specific PPE may include sleeve covers, Tyvek suits, disposable gowns, disposable hair nets or shoe coverings. See Chapter 9 for additional information on PPE.

Chapter 8: Animal Biosafety

Work with animals and biohazards presents unique hazards, such as generation of aerosols, bites and scratches, and shedding of agents, all of which is considered during the risk assessment for animal work involving biohazards or rDNA. Work with infectious agents or rDNA in animals is classified as Animal Biosafety. There are four Animal Biosafety levels (ABSL) that are required for the use of experimentally infected animals housed in research facilities, animals administered rDNA, or maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In general, the biosafety level recommended for working with infectious agents in vivo is the same as that for working with the agents in vitro. Animal biosafety level is determined by the Biosafety Officer.

Animal Biosecurity

Animal Biosecurity is a set of preventative measures designed to reduce the risk of transmission of infectious agents among animals or between animals and humans. This designation includes agents that may not be an infectious risk to humans but are animal pathogens, as well as zoonotic agents that have the potential to spread among both animals and humans. Evaluation includes work practices, PPE, risk assessments and a housing requirement designed to reduce the risk of transmission within an animal colony.

Levels

Animal biosafety levels, similar to biosafety levels, provide increasing protection to personnel and the environment. These levels are the general minimum requirement for work with exposed laboratory animals.

ABSL-1

ABSL-1 is appropriate for work with animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present a minimum hazard to personnel and the environment.

ABSL-2

ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. ABSL-2 also includes work involving some viral vectors and rDNA use, as well as the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. It builds upon the practices, procedures, containment equipment and facility requirements of ABSL-1. Work at ABSL-2 requires a protocol that has been approved by both the IBC and IACUC prior to the initiation of any work.

ABSL-3

ABSL-3 is appropriate for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 requirements build upon the foundation of lower level practices, procedures, containment equipment and facility requirements of ABSL-2. Work at ABSL-3 requires a protocol that has been approved by both the IBC and IACUC prior to the initiation of any work.

ABSL-4

ABSL-4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal and for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. ABSL-4 work is not conducted at UMass Amherst.

ABSL Work Practices

ABSL work practices build on BSL work practices (see Table 2 in Chapter 3) but incorporate specific animal-related items. All BSL-1, 2 or 3 work practices, including but not limited to those addressing decontamination, BSC use, physical containment, handwashing and pipetting, must be followed for the equivalent ABSL.

Designated ABSL-2 housing and procedure spaces exist within the vivarium facilities. Animal Care Services (ACS) designated PPE must be worn in all ABSL-2 facilities. PPE is provided by ACS for animal housing locations within the vivarium, and signs indicating the required PPE are posted in ABSL-2 spaces. If different PPE practices are employed from those posted, the PPE must be discussed and outlined in the associated IBC protocol and with the ACS. For animal work that is done in laboratory spaces, BSL-2 and ABSL-2 work practices must be followed. If animal work is done outside the vivarium, transport of ABSL-2 animals must be done using appropriate containment measures, and carcasses and caging/bedding must be returned to the ACS for appropriate disposal.

Training

The Animal Care Services team offers rodent handling training to show personnel basic techniques, as well as specific techniques or consultations on various equipment or practices. Additional training is available (Chapter 6).

Content	Where
Biosafety for Laboratory Staff	OWL (classroom and online)

Bloodborne Pathogens	OWL
BSL-3 Training	In-person
Laboratory Safety	OWL (classroom and online)
Fire Safety	OWL (classroom)
Autoclave Safety	OWL (online)
Animal User Training	Research Admin & Compliance (classroom)
Working with the IACUC	CITI (Research Admin & Compliance) online
Livestock Biosafety	Upon request
Poultry Biosafety	Upon request
Field Research Safety	Module available in OWL or by request

Tissue Only Protocols

Use of some animal tissues requires an IACUC protocol due to occupational health concerns, even if the tissues are a by-product of other IACUC -approved studies, are obtained from a slaughterhouse, or are commercially available as standard “off-the-shelf” products. To determine if your use of tissue requires a protocol, please contact IACUC. If it is confirmed you will need a protocol, you can file a tissue-only protocol. Contact IACUC protocol determination and requirements.

Zoonoses

Zoonotic agents are those that can be transmitted between species; zoonoses that can be transmitted between humans and animals fall under the auspices of Animal Biosafety. Human exposure can occur through multiple routes, including bites, scratches, aerosol droplets, mucosal secretions, feces or urine. For animals inoculated with agents that are infectious but not necessarily to humans, there is the concern of transmission among a susceptible animal colony, and these agents fall under the auspices of Animal Biosecurity. While many laboratory animal species are bred to be free of zoonoses, there are zoonotic agents associated with laboratory animals, some of which can pose a risk to humans or other animals in the colonies. Additionally, humanized animals (those administered or implanted with human tissues or cells) capable of supporting replication of human agents, animals with altered genotypes resulting in new or increased susceptibility to infectious agents, and animals with altered immune systems (such as severe combined immunodeficient, or SCID, mice) require specific risk assessments for zoonotic issues.

Wild animals pose additional zoonotic risks, as their health history is unknown. Infectious agents or animals that pose a zoonotic threat are classified at the appropriate ABSL or Animal Biosecurity Level and are housed as such.

Field Work

Field work with or around animals may benefit from review by Biosafety. In addition to items outlined in Chapter 7, field work with animals raises issues. PPE, zoonoses, procedures and practices should be discussed during individual risk assessments conducted for each work scenario. Discussion and considerations take into account what is being done and where, the physical setting, the use or proximity to animals and potential zoonotic or other hazards, access to engineering controls both in the field and in the lab, safety procedures in the field, awareness of surroundings, and issues specific to the region or work being conducted. Use and housing of any animals that are brought back to campus must adhere to IACUC standards for quarantine, be reviewed for zoonotic risks, and may require permits depending on the species and import status.

Chapter 9 Safety: Practices and Equipment

The creation of a safe working environment for all personnel and ensuring that the work being done does not impact the environment is the duty of all individuals working in laboratories. Good work practices, facility design, equipment training, and protective clothing should all combine to provide a safe work environment.

There are obvious dangers to working with infectious agents/rDNA/sNA. Pathogens can infect a host through a number of routes, and it is important to be aware that a laboratory-acquired infection may not follow the same route as a naturally occurring one. The following are some of the more common accidents that can result in infection:

As aerosols are important sources of infection, care should be taken to reduce the extent of their formation and dispersion. Hazardous aerosols can be generated by many laboratory operations, e.g. blending, mixing, grinding, shaking, stirring, sonicating and centrifuging of infectious materials. Even when safe equipment is used, it is best to carry out these operations in an approved biological safety cabinet whenever possible.

The following table lists biosafety equipment and the hazards mitigated.

Equipment	Hazard Corrected	Safety Features
BSC Class II A2	Aerosol and spatter	• Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air • Provides product protection
Spatter shield	Spatter of materials	• Forms screen between operator and work
Pipetting aids	Hazards from pipetting by mouth, e.g. ingestion of pathogens, inhalation of aerosols produced by mouth suction on pipette,	• Ease of use • Controls contamination of suction end of pipette, protecting pipetting aid, user and vacuum line • Can be sterilized

	blowing out of liquid or dripping from pipette, contamination of suction end of pipette	• Controls leakage from pipette tip
Micro incinerator	Spatter from transfer loops	• Shielded in open-ended glass or ceramic tube • Heated by gas or electricity
Disposable loops	Spatter from transfer loops	• Disposable, no heating necessary
Leak-proof vessels for collection and transport of infectious materials	Aerosols, spills, leaks	• Leak-proof construction with latching lid or cover • Durable • Autoclavable
Sharps disposal containers	Puncture wounds	• Autoclavable • Robust, puncture-proof
Transport containers	Release of microorganisms	• Robust • Watertight primary and secondary containers to contain spills • Absorbent material to contain spills
Autoclaves	Infectious material (made safe for disposal or reuse)	• Approved design • Effective heat sterilization
Screw-capped vials	Aerosols and spillage	• Effective containment
Vacuum line protection	Contamination of laboratory vacuum system with aerosols and overflow fluids	• Cartridge-type filter prevents passage of aerosols (particle size 0.45µm) • Overflow flask contains appropriate disinfectant. • All components are autoclavable
Homogenizers, shakers, blenders and sonicators	Aerosols and spillage	• Only equipment designed for laboratory use should be used • Use of Risk Group 3 organisms requires that loading and unloading this equipment takes place within the biosafety cabinet.

Universal Precautions

The concept of Universal Precautions is to treat all human/primate blood and other body fluids, tissues and cells as if they were known to be infectious for BBPs. Universal Precautions includes frequent handwashing, no mouth pipetting, no food or drink in the lab and proper disposal of biohazardous/medical waste, as well as the use of engineering controls and Personal Protective Equipment (PPE). Engineering controls include items such as biosafety cabinets, ventilation systems, closed top centrifuge rotors, etc.; these are the primary methods to control exposure. PPE such as gloves, lab coats, eye protection, face shields or others must be selected and used as appropriate. See Chapter 4 for additional information.

Personal Protective Equipment

Personal protective equipment (PPE) is a necessary part of laboratory safety in addition to engineering controls (i.e., laboratory ventilation and Biosafety Cabinets) and good work practices. When properly selected and used, personal protective equipment can be effective in minimizing individual exposure.

PI's have the primary responsibility for implementing the PPE Program in their work area by ensuring that workplace hazards have been evaluated, that the appropriate PPE is available, and that employees have received the necessary training.
This involves:

- Wearing PPE as required per the risk assessment
- Attending site-specific PPE training sessions
- Cleaning and maintaining PPE as trained
- Informing the PI/supervisor of the need to repair or replace PPE

Due to intrinsic hazards such as performing injections with biological agents or necropsies on infected animals, special attention should be given to using puncture and cut resistant gloves.

Lab Coat Program

Lab coats are a key element of personal protective equipment in laboratories that not only protects the skin from exposure to hazardous materials, it protects clothing from becoming contaminated and keeps hazardous materials from exiting the lab with the individual. Lab coats should be periodically laundered to remove possible contaminants and maintain them in hygienic condition. Home and public laundry facilities must not be used to clean lab coats. The lab coat management program provides a means to acquire properly fitting lab coats and the opportunity to launder lab coats as needed through an appropriate laundering service.

EH&S has a purchase program for lab coats through Fisher Scientific and separate laundering with Belmont Laundry in Springfield, MA. Lab coats are purchased in bulk and are set-up with the UMass logo and laundering tag ready to be assigned to an individual. When an order is placed for a particular type and size of coat, a name label is applied and the coat is delivered to the lab coat pick up/drop off room in the building where the lab is located. The cost of the lab coats and set-up is recharged to the faculty/supervisor's department or school. An individual can send their coat for laundering by placing it in the hamper in the lab coat pick-up/drop-off room in their building. EH&S collects the coats and sends them out for laundering every Friday. They are returned the following Friday and redelivered to the lab coat pick-up/drop-off location shortly after. The cost of laundering is covered by EH&S and is not recharged to the department or school.

What types of lab coats are available and which is appropriate for my use?

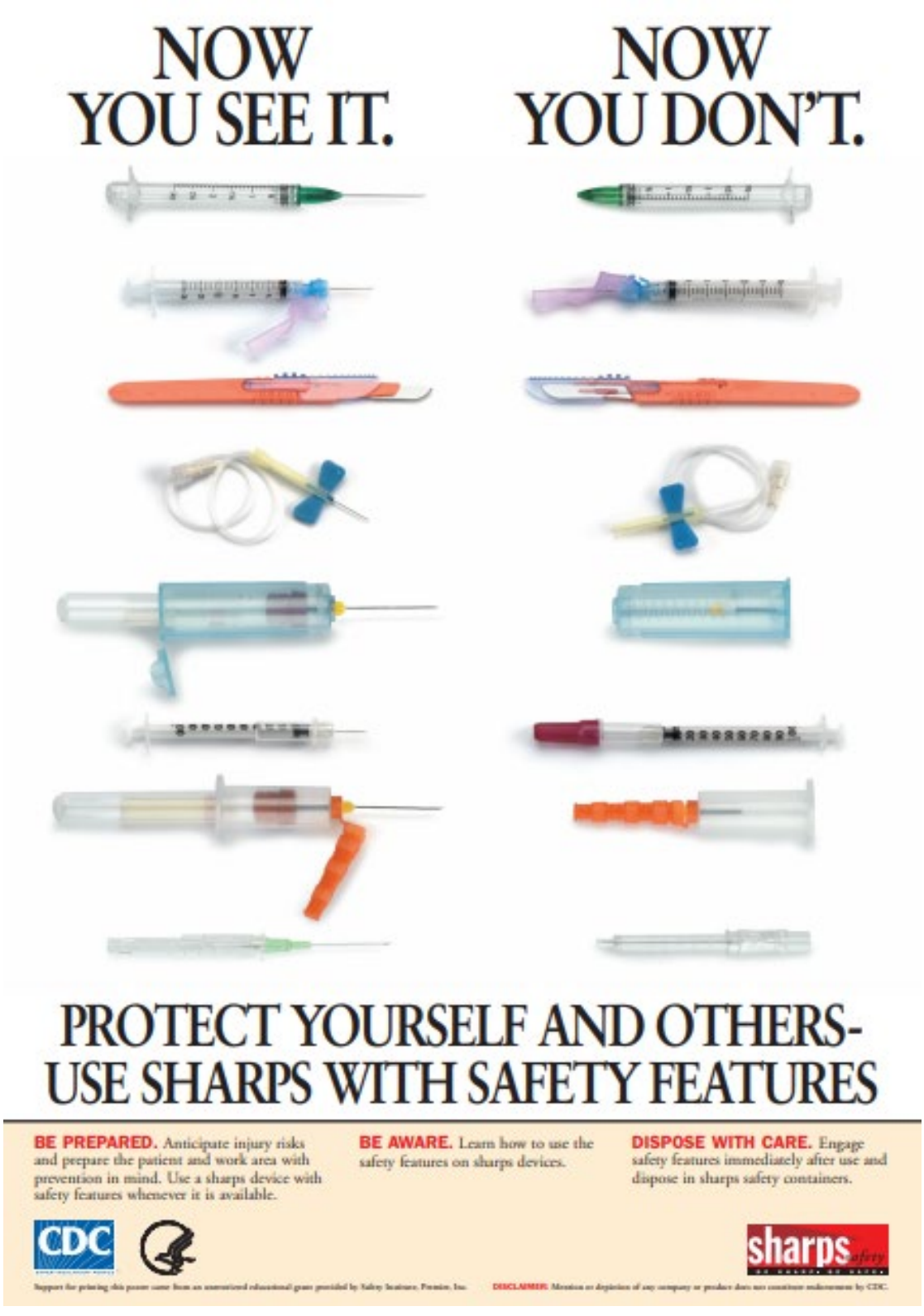
Three different styles of white poly/cotton lab coats are available and are suitable for most labs. Sleeves with knit cuffs are a good choice for individuals working with biological materials.

In addition, flame resistant treated 100% cotton lab coats in blue are available for those whose work in labs involves larger quantities of flammable liquids. Special requests can be made for lab coats made of Nomex™ which is a flame resistant fabric. These coats offer a better flame resistance over the long-term as there is no treatment that can be washed away over time. The flame resistance is due to the type of fabric rather than a coating. In addition, special requests can be made for lab coats made of Nomex™ with an enhanced chemical protection coating for work with particularly hazardous chemicals such as hydrofluoric acid. Sizes available vary by type of coat but range from XXS to 5XL with long sizes available.

To obtain more information, please go to:
https://ehs.umass.edu/lab-coat-management-program

Safety Engineered and Needleless Sharps

Manufacturers have developed “engineered sharps” for commonly used items (e.g. scalpels, syringes, needles) that have various mechanical devises to vastly decrease the occurrence of injuries due to sharps. OSHA recommends any laboratory using human or primate blood, blood products, cell lines, tissues or other potentially infectious materials to use needleless systems/and or engineered sharps (Figure below). If a PI/supervisor decides that a noncompliant sharp is necessary for a certain procedure, the reason must be documented in a risk assessment.


NOW
YOU SEE IT.
NOW
YOU DON'T.
PROTECT YOURSELF AND OTHERS-
USE SHARPS WITH SAFETY FEATURES
BE PREPARED. Anticipate injury risks and prepare the patient and work area with prevention in mind. Use a sharps device with safety features whenever it is available.
BE AWARE. Learn how to use the safety features on sharps devices.
DISPOSE WITH CARE. Engage safety features immediately after use and dispose in sharps safety containers.
CDC
sharps safety

Biological Safety/Biosafety Cabinets

Biological safety cabinets (BSC) are designed to provide three types of protection:

- Protection for personnel from material inside the cabinet
- Protection for the material inside of the cabinet from personnel and the environment
- Protection for the environment from the material inside of the cabinet

There are three types of BSCs: Class I, II, and III.

Class I

Class I cabinets are designed to provide personnel and environmental protection only. The material (research experiment) inside the cabinet is not protected and thus subject to contamination. *The use of Class I BSC is not advised at UMass Amherst*; consult with Biosafety if you feel you need to purchase one.

Class II

Class II cabinets meet requirements for the *protection of personnel, product and the environment.* There are four types of Class II cabinets (A2, B1, B2, and B3), each differentiated according to the method by which air volumes are recirculated or exhausted.

Class II, type A: The Class II, type A biosafety cabinet does not have to be vented, which makes it suitable for use in laboratory rooms which cannot be ducted. This cabinet is acceptable for use of low to moderate risk agents in the absence of volatile toxic chemicals and volatile radionuclides. *The most common biosafety cabinet on campus is a Class II A2.*

Class II, type B1: The Class II, type B1 biosafety cabinet must be vented. 30% of the air is exhausted from the cabinet while 70% is recirculated back into the room. This cabinet may be used with etiologic agents treated with minute quantities of toxic chemicals and trace amounts of radionuclides required as an adjunct to microbiological studies if work is done in the directly exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.

Class II, type B2: The Class II, type B2 biosafety cabinet must be totally exhausted. 100% of the air from the cabinet is exhausted through a dedicated duct. This cabinet may be used with etiologic agents treated with toxic chemicals and radionuclides required as an adjunct to microbiological studies.

Class II, type B3: The Class II, type B3 biosafety cabinet must be vented. 70% of the air is exhausted from the cabinet while 30% is recirculated. This cabinet may be used with etiologic agents treated with minute quantities of toxic chemicals and trace quantities of radionuclides that will not interfere with work if recirculated in the downflow air.

Class III biosafety cabinets (glove boxes) provide maximum microbiological containment to the environment and to the worker. Access to the interior of the cabinet is through a double-door pass-through "interchange" box. Both supply and exhaust air are HEPA filtered. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet. Class III cabinets are usually only installed in maximum containment laboratories that have controlled access and require special ventilation.

Aspiration of Liquid Waste

A vacuum flask system is required to provide protection to the central building vacuum system or vacuum pump and to personnel who service the equipment. Figure 4 illustrates a proper set-up for handling liquid waste. Additionally, Flasks A and B must be placed in secondary containment. Please consult with Biosafety if you have any questions.

Figure 4. Liquid Waste

The left suction flask (above left, A) is used to collect the contaminated fluids into a suitable decontamination solution; an example setup of this is photographed in (middle), an alternate setup shown in (right).

Use of Open Flames in Biosafety Cabinets/Tissue Culture Hoods:

Not recommended

Background

Early microbiologists had to rely on open flames to ensure sterility while engaging in certain techniques on the bench. With the advancement of modern technology, including the introduction of the Biosafety cabinet, the use of an open flame is almost always *no longer necessary*. In fact, the use of open flames in a Biosafety cabinet:

- Disrupt the air flow, compromising protection of both the worker and the work

- Cause excessive heat buildup, may damage HEPA filters and/or melt the adhesive holding the filter together, thus compromising the cabinet's integrity,
- Present a potential fire or explosion hazard. Electrical components such as the fan motor, lights and electrical outlets are not designed to operate in flammable atmospheres, where a flash fire could be ignited by a spark.
- Inactivate manufacturers' warranties on the cabinet: cabinet manufacturers will assume no liability in the event of fire, explosion or worker exposure due to the use of a flammable gas in the cabinet. Additionally, the UL approval will automatically be void.

The Centers for Disease Control and Prevention (CDC) reports that "open-flames are not required in the near microbe-free environment of a biological safety cabinet" and create "turbulence which disrupts the pattern of air supplied to the work surface," jeopardizing the sterility of the work area. This is also the recommendation of the World Health Organization (WHO) as well as the major Biosafety cabinet manufacturers.

The use of such devices is not only extremely dangerous, but can also inactivate manufacturer's warranties. There are many alternatives to the use of burners: micro-incinerators, disposable tissue culture supplies, etc.

Solutions

- Follow good BSC work practices
- Remove Bunsen burners and/or replace with alternative technology such as electric incinerators Use disposable loops, spreaders, and other instruments
- Autoclave instruments such as tweezers, scissors and scalpels
- Reduce the amount of flammable chemicals in the cabinet. Use only enough alcohol for one day's work
- Use alcohol to sterilize any glass, etc. that is being used. Allow to evaporate before opening or dry with a tissue
- If it is deemed absolutely necessary for the work being done, use a pilotless burner or touch-plate micro-burner to provide a flame on demand.

BSC Best Practices:

- Turn on BSC and let run for 5 – 10 minutes before using
- Wipe down cabinet surfaces with appropriate disinfectant
- Arrange work surface from "Clean" to Dirty", keeping air grilles clear of materials
- Disinfect and remove all materials from cabinet after use
- Leave BSC running for 5–10 minutes after use
- Wipe down cabinet surfaces with appropriate disinfectant
- Clean the BSC on a routine basis to eliminate contaminated residue buildup. **All BSCs have a tray under the work space** (see photo below) where the return air passes on its way to the HEPA filter. Any debris present in the BSC will eventually be carried along

with this air and may settle out in this tray. Any spilled materials may also end up in this tray. Over time, the built up contaminants may start to “grow”, which could lead to product contamination.

Class II A2 BSC (above) with work tray removed for proper cleaning.

Installation and Maintenance of BSCs

Installation of cabinets must be done by certified professionals. Notify EH&S of all new BSC's and we maintain a campus inventory for this equipment. UMass Amherst has a contract with a certified company for installation, decontamination, repairs and any other needs that may arise. Arrangements and payment for any of the above work must be scheduled by the PI or the Department. Please coordinate with Biosafety so that we may track and manage the reports.

Cabinet certification is arranged by the Biosafety Department on an annual basis. EH&S pays for the annual certification of Class II A2, Class II B2, Animal Transfer Stations, and Bedding Disposal Stations.

Location of BSC's in Labs

A BSC air curtain is very delicate and provides the only barrier between the inside (potentially infectious aerosols) and the outside (where personnel are) air. Air-flow turbulence from both inside and outside of the BSC risks breach of containment; as such, cabinets should be located away from doors, high traffic areas and building HVAC systems (vents). The Biosafety program can assist in evaluating the best placement for a BSC to protect both the material and the researchers.

Relocate or Dispose of a Biological Safety Cabinet

Because of the size and weight of BSC's, there are specific considerations that apply to moving BSC's in order to assure that this is achieved safely and without spreading contaminants.

- Gas decontamination is necessary under certain circumstances in order to prevent spread of infectious contaminants and protect personnel involved in BSC disposal roles. These include:
    - The BSC is being moved out for disposal (i.e., via demolition/donation to another lab).
    - The BSC that has been used for work with infectious agents (i.e., those that are considered Risk Group 2 or higher, or USDA permitted) is being moved from the current lab space to a different lab space.
    - The BSC is being moved to a different lab space and has an incomplete history of use for the currently installed HEPA filters.

NOTE: A copy of the BSC certifier's decontamination report must be posted on the BSC or it cannot be moved!

Signs and Hazard Communication

All laboratories must have a lab door card on the outside of the door indicating that biohazardous material is used within the room. Investigators who are using BSL-2 or 3 agents or

rDNA/sNA are required by the NIH to post a sign that incorporates the universal biohazard symbol on the outer laboratory door. The sign must include the agent name, Biosafety level, and specific requirements for entry, the PI's name and spaces for phone numbers of laboratory staff in case contact must be made. Lab Door cards are available through EH&S.

Red-orange coded biohazard labels must be placed on storage freezers, refrigerators, on any laboratory equipment used with BSL-2 or 3 agents, shipping containers, medical waste containers or any surface which may be reasonably anticipated to encounter surface contamination from biohazardous materials. These labels are available through EH&S.

Exposures

For any Exposure or Incident, the following steps shall be taken:

Step 1: Care for Personnel

- If there has been a needlestick/puncture, wash the affected area with antiseptic soap and warm water for 15 minutes.
- For a mucous membrane exposure, flush the affected area for 15 minutes using an eyewash.

Step 2: Medical Attention

- If medical attention is needed, go to University Health Services (UHS) (non-life threatening incidents) or to Cooley Dickenson Hospital Emergency Department for medical emergencies or after hours. If a spill has occurred, contain and initiate clean up (see below).

Step 3: Notification

- Notify PI, manager, or supervisor
- Go to UHS for treatment ASAP (within 2 hours)

Step 4: Reporting

- Complete a Lab Incident Report and submit to EH&S within 24 hours https://ehs.umass.edu/lab-incidents-and-lab-incident-report-form
- Complete a "Notice of Injury" form if you are an employee. This form is submitted to Human Resources to initiate Workman's Compensation Insurance benefits. Contact the Human Resource Worker's Compensation Specialist at: 413-545-6114, and ask for an NOI form. The NOI must be completed by the injured employee and signed by his/her supervisor. Return the NOI to HR within 48 hours of the incident.

Spill Response

The following procedures are provided as a guideline to biohazardous/rDNA/sNA spill cleanup. If the spill is considered too large or too dangerous for laboratory personnel to safely clean up, secure the entire laboratory and call EH&S (413-545-2682) immediately for assistance.

Bleach is recommended as a standard disinfectant; however, other disinfectants may be used, provided they are effective against the particular agents. Disinfectants must be used at the appropriate dilution for the required minimum contact time.

Inside the Biosafety Cabinet

1. Wait at least five minutes to allow the BSC to contain aerosols.
2. Wear laboratory coat, safety glasses and gloves during cleanup.
3. Allow BSC to run during cleanup.
4. Wipe up spillage with paper towels. Place the contaminated towels in biohazardous waste container. Do not place your head in the cabinet to clean the spill; keep your face behind the view screen.
5. Apply disinfectant to all surfaces inside the BSC and allow appropriate contact time for disinfectant being used.
6. Wipe the walls, work surfaces, and any equipment in the cabinet with paper towels. Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures. Place contaminated reusable items in biohazard bags or autoclavable pans with lids before autoclaving. Expose non-autoclavable materials to disinfectant (appropriate contact time) before removal from the BSC.
7. Remove protective clothing and segregate for disposal or cleaning.
8. Run BSC 10 minutes after cleanup before resuming work or turning BSC off.
9. Wash hands with soap and water prior to leaving area.

NOTE: If the spill overflows the drain pan/catch basin under the work surface into the interior of the BSC, notify EH&S. A more extensive decontamination of the BSC may be required.

In the laboratory, outside the Biosafety Cabinet

1. Evacuate Room: insure all personnel are accounted for and that doors are closed. Put notice on door informing personnel of spill and not to enter. Allow spill to settle (30 min.).
2. Assemble clean-up materials (disinfectant, paper towels, biohazard bags and forceps).
3. Put on appropriate PPE, including lab coat, shoe covers, gloves and eye/face protection.
4. Initiate cleanup with disinfectant as follows:
    - Place paper towels or other absorbent material over spill area
    - Allow liquids to absorb
    - Remove the contaminated materials and place in biohazardous waste

- Carefully pour disinfectant over the spill area and cover with paper towels. Avoid splashing or generating aerosol droplets.
- Allow disinfectant to remain in contact with spill area for at least 10 minutes
- Apply more paper towels to wipe up spill
- Dispose of all towels or absorbent materials using appropriate standard waste disposal procedures (these towels are already soaked with disinfectant and considered non-biohazardous at this point. If any sharp objects are present, use forceps and discard in a sharps container.
- Remove protective clothing and segregate for disposal or cleaning.
- Wash hands with soap prior to leaving area.

Inside a centrifuge

1. Clear area of all personnel.
2. Wait 30 minutes for aerosol to settle before attempting to cleanup spill.
3. If a spill is identified after the centrifuge lid is opened, carefully close the lid, evacuate the laboratory and close the laboratory door. Remain out of laboratory for at least 30 minutes. Put notice on door informing personnel of spill and not to enter.
4. Wear a laboratory coat, safety glasses and gloves during cleanup.
5. Remove rotors and buckets to nearest BSC for cleanup.
6. Thoroughly disinfect inside of centrifuge.
7. Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures.
8. Wash hands with soap prior to leaving area.

Outside the laboratory

1. To prevent a spill, transport labeled biohazardous material in an unbreakable, well-sealed primary container placed inside of a second unbreakable, lidded container (cooler, plastic pan or pail) labeled with the biohazard symbol, biosafety level and contact information.
2. Should a spill occur in a public area, do not attempt to clean it up without appropriate PPE.
3. Secure the area, keeping all people well clear of the spill.
4. If help is needed, call EHS at 413-545-2682 to assist in cleanup.
5. Stand by during spill response and cleanup activity and provide assistance only as requested or as necessary.
6. If you participated in cleanup, wash hands with soap upon leaving area.

Chapter 10: Transportation

Transportation of Biohazardous Goods on UMass Amherst Campus

Transport of biohazardous goods within the UMass Amherst campus requires the use of proper secondary containment. Secondary containers can be a variety of items but must be leak-proof and have tight fitting covers. All containers must be labeled with a biohazard sticker or label Use tertiary containment for transport from off-campus locations.

Shipping of Biohazardous Goods off Campus

Transport of biohazardous goods off campus requires training and certification prior to shipping. Federal (FAA, 49 CFR) and international agencies (ICAO, the branch of the United Nations that governs all international civil aviation matters, IATA, and the International Air Transport Association) have in place numerous regulations for shipping of dangerous goods by surface or air. Training is mandatory for shippers (the person sending out the package) and handlers (the people who transport the package) and is based on these regulations. Nonconformance of these regulations can result in a fine and/or imprisonment.

UMass Amherst has a shipping program that is to be used for all hazardous good shipped from this campus. Please refer to this site for additional information on several shipping topics: https://ehs.umass.edu/shipping-program-hazardous-materials

Export Controls Related to Biologicals and Toxins

The Commerce Department (https://www. commerce.gov/), along with other federal agencies, regulates shipping of biologicals and toxins outside the U.S. All select agents and many biological agents and toxins are controlled for export, and require US government authorization in the form of an export license before they may be shipped internationally.
UMass Amherst's Export Controls website:
http://www.umass.edu/research/compliance/research-safety-and-security/export-controls

Export Control Officer must be contacted before any export-controlled biological material or toxin is shipped abroad so that an export license can be obtained. Note: the export licensing process can take up to two months so plan well in advance. All other exports of biologicals need to be documented with the appropriate export certification signed by the responsible PI or researcher.

Import of Biohazardous Goods onto Umass Amherst Campus

The Federal Government, in its shipping and transportation standards, defines etiologic agents as microorganisms that cause disease in humans including the following: bacteria, bacterial

toxins, viruses, fungi, rickettsia, protozoans, parasites and prions. These disease-causing microorganisms may also be referred to as infectious agents or infectious substances, and the materials, such as body fluids and tissues that contain them, are referred to as infectious materials. When a package of infectious material is being imported into the United States, it must have an importation permit approved by the CDC. Organisms such as mosquitoes that might transmit infectious diseases to other humans are called vectors. Vectors may require permits from agencies such as the CDC, USDA or the Massachusetts Department of Public Health.

It is important to obtain a CDC permit PRIOR to requesting an etiologic specimen from a source outside the United States. The IBC will request that the Principal Investigator indicate the source of any agents used in experiments at UMass Amherst during the application process. If the investigator intends to obtain the agent from outside the United States, a copy of the CDC or other permit will be requested by the IBC as part of the IBC review of the application.

Items Requiring Permits

Etiologic agents

It is impractical to list all of the several hundred species of etiologic agents. In general, an import permit is needed for any infectious agent known to cause disease in humans. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts, molds, and prions. In some instances, agents which are suspected of causing human disease also require a permit.

Biological Materials

Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected of being infected with disease transmissible to humans require a permit under these provisions in order to be imported.

Animals

Any animal known or suspected of being infected with any disease transmissible to humans. Importation of turtles of less than 4 inches in shell length and all non-human primates requires an importation permit issued by the Division of Quarantine.

Insects

Any living insect, or other living arthropod, known or suspected of being infected with any disease transmissible to humans. Also, if alive, any fleas, flies, lice, mites, mosquitoes, or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.

Snails

Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either Centers for Disease Control or the Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.

Information regarding CDC permitting:

US CDC Import Permit Program tool: https://www.cdc.gov/cpr/ipp/etool.htm

Centers for Disease Control and Prevention
Import Permit Program
1600 Clifton Road NE, Mailstop A-46
Atlanta, GA 30333
Telephone: 404-718-2077
FAX: 404-471-8333
Email: importpermit@cdc.gov
www.cdc.gov/od/eaipp/
importpermit@cdc.gov

USDA/APHIS Permits

United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required for infectious agents of livestock and biological materials containing animal products, particularly livestock material.
https://www.aphis.usda.gov/aphis/ourfocus/importexport

Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials and suspensions of cell culture used to grow viruses or other etiologic agents and which contain growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal diseases into the U.S. Further information may be obtained at:
https://www.aphis.usda.gov/aphis/resources/permits or by calling the USDA/APHIS at 1-888-272-3181.

Letters of Authorization

After a review of an "Application to Import an Etiological Agent", the issuing officer may issue a "Letter of Authorization" rather than an importation permit. The Letter of Authorization is issued for materials that are judged to be non-infectious, but which might be construed to be infectious by U.S. Customs inspection personnel.

Letters of Authorization may be issued for items such as formalin fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine, or cerebrospinal fluid, or other tissues or materials of human origin when there is no evidence or indication that such materials contain an infectious agent.

A copy of a Letter of Authorization should be attached to the package, and also should be furnished to the courier or importation broker. Letters of Authorization are in effect for two years.

Export of Infectious Materials

The export of infectious material may require a license from the Department of Commerce.
Eastern Region http://bit.ly/2BAk4Ut
Western Region http://bit.ly/2B1Zz3J
To speak with a Department of Commerce export counsellor, call one of the following numbers:
(202) 482-4811 - Outreach and Educational Services Division (located in Washington, DC)
(949) 660–0144 - Western Regional Office (located in Newport Beach, CA)
Find local branch or e-mail your inquiry to the Export Counseling Division of the Office of Exporter Services at: ECDOEXS@bis.doc.gov

US Customs and Border Protection Database: https://help.cbp.gov/s/sidebar-top-5-import?language=en_US

International Traffic in Arms Regulations:
www.LearnExportCompliance.com/itar (updated monthly)

Commerce Control List:
www.learnExportCompliance.com/ccl (updated monthly)

Additional Resources:

- Centers for Disease Control and Prevention Public Inquiries/OHS
  Mailstop F05
  1600 Clifton Road
  Atlanta, GA 30333 U.S.A
  https://www.cdc.gov/biosafety/

- Centers for Disease Control and Prevention
  Import Permit Program - Frequently Asked Questions
  https://www.cdc.gov/phpr/ipp/faq.htm
- US Dept. of Transportation / Pipeline and Hazardous Materials Safety Administration
  https://www.phmsa.dot.gov/

Chapter 11: Waste and Decontamination

Waste

Biohazardous waste includes all laboratory waste that may contain any biohazardous material or were in contact with said material. Additionally, any blood or components of blood or body fluids are to be disposed of as biohazardous waste, as are human or non-human primate cell lines. All biohazardous waste must be disposed of in clear autoclave bags, NOT marked with the biohazard symbol; these bags must be secondarily contained in a puncture resistant outer container and covered with a tight fitting lid. UMass prefers the use of a step can for this purpose. Biohazard stickers must be present on all four sides of the step can and on the top of the lid.

In accordance with 105 CMR 480.00 the State Sanitary Code **Minimum requirements for the management of medical or biological waste (State sanitary code chapter VIII),** medical or biological waste is defined as:
Medical or Biological Waste.
Waste that because of its characteristics may cause, or significantly contribute to, an increase in mortality or an increase in serious irreversible or incapacitating reversible illness; or pose a substantial present potential hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed.

The following types of waste are identified and defined as medical or biological waste, and shall be subject to the requirements of 105 CMR 480.000:

1) **Blood and Blood Products**. Discarded bulk human blood and blood products in free draining, liquid state; body fluids contaminated with visible blood; and materials saturated/dripping with blood. Blood Products **shall not include**: feminine hygiene products. 105 CMR: DEPARTMENT OF PUBLIC HEALTH 480.010: continued
2) **Pathological 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; and discarded material saturated with body fluids other than urine. Pathological waste **shall not include**: Teeth and contiguous structures of bone without visible tissue, nasal secretions, sweat, sputum, vomit, urine, or fecal materials that do not contain visible blood or involve confirmed diagnosis of infectious disease.

3) **Cultures and Stocks of Infectious Agents and Associated Biologicals**. All discarded cultures and stocks of infectious agents and associated biologicals, including culture dishes and devices used to transfer, inoculate, and mix cultures, as well as discarded live and attenuated vaccines intended for human use, that are generated in:
    a) Laboratories involved in basic and applied research;
    b) Laboratories intended for educational instruction; or
    c) Clinical laboratories
4) **Contaminated Animal Waste**. Contaminated carcasses, body parts, body fluids, blood or bedding from animals known to be:
    a) Infected with agents of the following specific zoonotic diseases that are reportable to the Massachusetts Department of Agricultural Resources, Bureau of Animal Health pursuant to 105 CMR 300.140: African swine fever, Anthrax, Avian influenza – H5 and H7 strains and any highly pathogenic strain, Bovine spongiform encephalopathy (BSE), *Brucellulosis*, Chronic wasting disease of cervids, Foot and mouth disease, Glanders, Exotic Newcastle disease, Plague (*Yersinia pestis*), Q Fever (*Coxiella burnetti*), Scrapie, Tuberculosis, Tularemia (Francisella tularensis); or
    b) Infected with diseases designated by the State Epidemiologist and the State Public Health Veterinarian as presenting a risk to human health; or
    c) 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 puncture or cuts, including, but not limited to, all needles, syringes, lancets, pen needles, Pasteur pipettes, broken medical glassware/plastic-ware, scalpel blades, suture needles, dental wires, and disposable razors used in connection with a medical procedure.*
6) **Biotechnology By-product Effluents**. Any discarded preparations, liquids, cultures, contaminated solutions made from microorganisms and their products including genetically altered living microorganisms and their products.

*All sharps waste must be placed in an approved sharps container that is constructed of rigid, hard plastic and labeled with the universal biohazard symbol. Do not overfill the container. The lid of the sharps container must be shut prior to disposal. Glass pipettes that have come into contact with biohazardous waste must be discarded as sharps waste and not in broken glass containers.

Mixed Waste

Waste can often involve a mixture of medical and non-medical waste. The following is **not** categorized as **medical waste**:

- A mixture of medical waste and hazardous chemical waste is categorized as hazardous chemical waste and is subject to the statutes and regulations applicable to hazardous chemical waste.
- A mixture of medical waste and radioactive waste is categorized as radioactive waste and is subject to the statutes and regulations applicable to radioactive waste.
- A mixture of medical waste, hazardous chemical waste, and radioactive waste is categorized as radioactive waste and is subject to the statutes and regulations applicable to radioactive waste. Mixed chemical and biohazardous sharps waste will be placed into a sharps container for disposal and sent off site for incineration.

Animal Carcasses

After proper euthanasia of laboratory animals (IACUC Procedures), animal carcasses shall be placed in bags and brought to the appropriate vivarium freezer. Contaminated/ infected carcasses, including those administered rDNA/SNA, shall be placed in a red biohazard bag, and brought to the vivarium biohazard freezers. These carcasses are sent off site for incineration.

Autoclaves

Autoclave Use

Any laboratory medical/biological waste which is being autoclaved shall be placed in a CLEAR autoclavable bag devoid of biohazard symbols (available at the Fisher Stockroom in LGRT). The top of the bag shall be secured with indicator tape that will change color after the attainment of sterilization. Be sure that the clear autoclavable bag can withstand the autoclave cycle without melting. See autoclave procedures below.

A steam autoclave is a device designed to sterilize cultures, media, surgical instruments and medical waste. Autoclaves will sterilize on the basis of:

- Length of time in the cycle
- Temperature
- Pressure
- Steam

An autoclave is suitable for the treatment of certain types of medical waste but not all types.

The following items of medical waste must not be autoclaved:

- Items of medical waste which are mixed with volatile chemical solvents or radioactive materials (this waste must be handled as either chemical waste or radioactive waste)
- Pathological waste (pathological waste is handled as follows: animal carcasses are placed in a red bag and taken to the pathological waste freezers in the animal facility; human body parts are placed in a red bag and disposed of as medical waste without autoclaving.)

The following items of medical/biological waste can be autoclaved:

- Microbiological waste such as cultures of human or animal specimens from medical or pathological laboratories
- Cultures and stocks of microbiological specimens
- Waste contaminated with biohazardous materials such as contaminated paper towels or contaminated surgical gloves
- Plant materials
- Soil (double the amount of time in the autoclave to allow for the density of soil)

Considerations for effective autoclaving:

- Clear the strainer (drain) before each autoclave use. Obstructed drains may cause the autoclave to go into alarm and stop functioning.
- Do not overload the autoclave bag. The autoclave steam and heat cannot penetrate to the interior of an overloaded bag. The outer contents of the bag will be sterilized but the inner part of the bag will essentially be unaffected by the autoclave cycle
- Do not put sharp objects, such as broken glass in that can puncture the bag
- Do not overload the autoclave
- Do not mix autoclave bags and other items to be autoclaved in the same autoclave cycle. Liquid media requires a shorter cycle, often 15- 20 minutes while autoclavable medical waste requires a minimum of 60 minutes in order to be effectively sterilized
- To help ensure non-variability of sterilization, try to use a consistent loading pattern of materials within the autoclave (amount of material and location within autoclave)
- Record (in orange bio-waste log) autoclave conditions achieved for each cycle that is used to decontaminate medical waste. Validate autoclave effectiveness once every

month (biological indicators are a recommended method and are provided by EH&S free of charge). Retain records in an accessible location.
- Calibrate the autoclave's parametric devices annually in compliance with 105 CMR 480. The parametric devices measure the time, temperature and pressure of the autoclave.

Safety considerations for autoclave users:

- Wear personal protective equipment including heat-resistant gloves, goggles or safety glasses and a lab coat.
- Use caution when opening the autoclave door. Allow superheated steam to exit.
- Use caution when handling a bag in case sharp objects have been inadvertently placed in the bag. Never lift a bag from the bottom of the bag to load into the chamber. Handle the bag from the top.
- Watch out for pressurized containers. Superheated liquids may spurt from sealed containers. Never seal a container of liquid with a cork that may cause a pressurized explosion inside the autoclave.
- Agar plates will melt and the agar will become liquefied. Avoid coming in contact with this molten liquid. Use a secondary autoclavable tray to catch any potential leakage from the bag that would otherwise leak into the autoclave.
- Glassware may crack or shatter if cold liquid comes in contact with this superheated glassware. If glass breaks in the autoclave, use tongs, forceps, or other mechanical means to recover the fragments; make certain that the autoclave has been cooled down to avoid surface burns.
- Use an absorbent liner for glass vessels containing liquid. Never put autoclave bags or glassware directly in contact with the bottom of the autoclave.

To autoclave waste, follow the below procedures:

- Place waste as generated in an autoclavable clear bag
- Put autoclave tape loosely around the top of the bag and place the bag in a secondary container such as an autoclave pan
- Set the cycle for 60 minutes, 121 degrees Celsius at 15 PSI (or alternate required conditions depending on waste, e.g.: soil is 2 hours minimum)
- Document the conditions achieved during the cycle in the orange autoclaved waste logbook.
- After autoclaving, the sterilized clear bag must cool. Place a completed "Autoclaved Waste" sticker on the autoclaved bag. This bag is then placed into a solid color trash bag and disposed of as regular trash.
- A variety of factors must be taken into consideration prior to purchasing an autoclave; additional information concerning autoclave purchases is available from Facilities and Campus Services.

Decontamination

General Spill Disinfection Procedure

- Put on proper PPE
- Place an absorbent material (paper towel, bench diaper) over the contaminated surface and allow the liquids to absorb, this will prevent spread of contamination. Remove these materials and place them in a biohazardous waste container.
- Next, add liquid disinfectant to the area where the spill was.
- Allow sufficient contact time after applying the disinfectant. If the contact time is too brief, the surface will not be thoroughly disinfected.
- When cleaning a spill of concentrated material or if the disinfectant must act on an uneven surface, allow extra time for the disinfectant to act.
- Avoid using concentrated or undiluted solutions of your disinfectant to "speed up" the inactivation process. The surface that is being disinfected may be adversely affected by strong chemicals. This is especially significant when working with bleach, which is a very strong corrosive. Some disinfectants will leave a residue of chemicals behind.
- Rinse the cleaned area with distilled water to avoid adverse effects on your experiment. This is especially important in tissue culture rooms where a cell line can be wiped out by disinfectant residue left on equipment.

Disinfectant Selection

Disinfectant selection is based on several factors.

- What is the target organism that you wish to inactivate?
- What are the physical characteristics of the surface which will be disinfected? (Porous surfaces may absorb disinfectants; some disinfectants may corrode metal surfaces).
- How long will the contact time be between the disinfectant and the target organism? High concentrations of biological organisms may require longer contact times.

Note that the disinfection of prions and prion-like proteins must follow specific guidelines and are discussed in "Agent Specific Treatment".

It is important to note that "bleach" is a very common and effective disinfectant, and is not stable at dilute concentrations; working dilutions of sodium hypochlorite should be made daily from a stock solution. Working solutions of 10% bleach (1:10 dilution of household bleach in water) are effective in most situations.

Alcohol based disinfectants will also evaporate over time and should be made up at appropriate intervals.

The following list of disinfectants, their efficiencies, contact times and recommended dilutions are general guidelines—please follow specific manufacturer's recommendations if available.

Quaternary Ammonium Compounds are commonly used in floor cleaning solutions. Quaternary ammonium compounds are effective in inactivating most vegetative bacteria, fungi, and lipid containing viruses. Quaternary ammonium compounds are NOT effective when used to disinfect Mycobacterium tuberculosis (TB), bacterial spores, and many viruses such as HBV.

- Recommended contact time: 10 minutes
- Recommended Working Dilution: 0.1-2.0%
- Recommended for: cleaning optical instruments and administrative areas in the vicinity of a laboratory

Ethanol is commonly used on equipment whose surfaces are susceptible for corrosion if other disinfectants are applied. Ethyl alcohol is effective in inactivating most vegetative bacteria, fungi, and lipid containing viruses. Ethanol is NOT effective when used to disinfect HBV, Mycobacterium tuberculosis (TB) and bacterial spores.

- Recommended contact time: 10 minutes
- Recommended Working Dilution: 70-85%
- Recommended for: Stainless steel surfaces.

Phenolics are commonly used to decontaminate surfaces such as lab bench tops. Phenolics are effective in inactivating vegetative bacteria, fungi, TB, lipid containing viruses and have some effect on HBV. However, phenolics will not inactivate bacterial spores.

- Recommended contact time: 10 minutes
- Recommended Working Dilution: 1.0-5.0%
- Recommended for: an alternative to bleach as a broad-spectrum disinfectant for bench tops, floors, and metal surfaces. Phenolics will not corrode metal surfaces as readily as bleach.

Hydrogen peroxide works by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA, and other essential cell components. Catalase, produced by aerobic organisms and facultative anaerobes that possess cytochrome systems, can protect cells from metabolically produced hydrogen peroxide by degrading hydrogen peroxide to water and oxygen. This defense is overwhelmed by the concentrations used for disinfection.

- Recommended contact time: 30 minutes
- Recommended Working Dilution: 3%
- Recommended for: biosafety cabinets, dental equipment, bench tops, floors and lab equipment in general. Vaporized hydrogen peroxide has been shown to be effective as a room sterilant as well as on large pieces of equipment (BSC).

Iodine-containing compounds or iodophors are commonly used to decontaminate metal surfaces or equipment. Iodophors are effective in inactivating vegetative bacteria, fungi, TB and lipid containing viruses and have some effect on HBV. However, iodophors will not inactivate bacterial spores.

- Recommended contact time: 10 minutes
- Recommended Working Dilution: 25-1600 ppm, 0.47%
- Recommended for: biosafety cabinets, dental equipment, bench tops, floors and lab equipment in general

Chlorine compounds such as bleach are commonly used in the lab because of the relative ease in accessibility and low cost. Chlorine (hypochlorite) compounds are effective in inactivating vegetative bacteria, fungi, lipid and non-lipid viruses, *Coxiella burnetii* and TB. Chlorine compounds have some effect in inactivating bacterial spores.

- Recommended contact time: 30 minutes
- Recommended Working Dilution: 500 ppm (1:10 dilution of household bleach, 5% hypochlorite ion)
- Recommended for: floors, spills (inactivating liquid specimens), bench tops and contaminated clothing. Do not use bleach on electronic equipment, optical equipment or unpainted stainless steel

Paraformaldehyde and formaldehyde were often used to decontaminate large pieces of laboratory equipment, such as biosafety cabinets (but only by professionals!). Paraformaldehyde/formaldehyde will inactivate vegetative bacteria, fungi, lipid and non-lipid viruses, HBV, TB, *Coxiella burnetii*, and bacterial spores. However, paraformaldehyde and formaldehyde are registered carcinogens i and are very toxic to use without the accessibility of a vented fume hood and/or personal protective equipment. **Do not use paraformaldehyde or formaldehyde in the lab to decontaminate equipment.** The approved biosafety cabinet contractor will use vaporized hydrogen peroxide to decontaminate your biosafety cabinet prior to changing the HEPA filters. Be sure to avoid using the biosafety cabinet while this operation is in effect.

Glutaraldehyde is often used to disinfect hospital instruments. Glutaraldehyde will inactivate vegetative bacteria, fungi, lipid and non-lipid viruses, HBV, TB, *Coxiella burnetii*, and bacterial spores. However, glutaraldehyde is very toxic to use without the accessibility of a vented fume hood and/or personal protective equipment. **Do not use glutaraldehyde in the lab to decontaminate equipment.**

Ethylene Oxide is often used to disinfect hospital instruments. Ethylene oxide will inactivate vegetative bacteria, fungi, lipid and non-lipid viruses, HBV, TB, *Coxiella burnetii*, and bacterial spores. However, Ethylene oxide is a registered carcinogen and is very toxic to use without

mechanically generated ventilation exhaust and personal protective equipment. **Do not use ethylene oxide in the lab to decontaminate equipment.**

Bleach Solutions (sodium hypochlorite)

Hypochlorite solutions are classified as irritants and corrosives. Undiluted bleach solution is corrosive to stainless steel, and thorough rinsing must follow its use in the BSC and stainless steel sinks to remove the residue. Do not autoclave bleach.

- Never mix different chlorine solutions or store them with cleaning products containing ammonia, ammonium chloride, or phosphoric acid. Combining these chemicals could result in release of chlorine gas, which can cause nausea, eye irritation, tearing, headache, and shortness of breath. These symptoms may last for several hours. A worker exposed to an unpleasantly strong odor after mixing of a chlorine solution with a cleaning product should leave the room or area immediately and remain out of the area until the fumes have cleared completely.
- To be an effective disinfectant, working bleach solutions must contain >0.5% but <2% sodium hypochlorite. Hypochlorite concentration in household bleach varies by manufacturer. Many household bleach solutions contain 5.25% sodium hypochlorite, and a 1:10 dilution (5,000 ppm Cl) will produce a 0.525% hypochlorite solution. Use of bleach solutions with lower hypochlorite concentrations might not provide the proper level of disinfection. Prepare a fresh 1:10 household bleach solution daily.

Agent Specific Treatment

Prions and Prion-like Proteins

Anyone working with prions, prion-like proteins or other Spongiform encephalopathies that may be present in brain tissue must call the Biosafety Officer, 413-545-2682. Slow viruses such as Kuru are included in this group. Prions and prion-like proteins (see Chapter 4 for a definition of prion- like proteins) are highly resistant to conventional decontamination, and laboratories are strongly encouraged to use only disposable equipment. Specific procedures for decontamination and disposal must be followed when working with prions and prion-like proteins. Contact Biosafety for further information, or visit the following sites for information on protocols:

World Health Organization (WHO) Infection Control Guidelines

Centers for Disease Control (CDC) Prion Diseases

Biosafety in Microbiology and Biomedical Laboratories (BMBL)

Chapter 12: Lab Deactivation & Equipment Disposal

Items for Lab Deactivation

Laboratories which utilize biological materials must notify the Biosafety program prior to terminating work to ensure that the laboratory has been decontaminated and that the biological material has been secured or properly disposed. If the Principal Investigator intends to cease work, he or she must notify Biosafety prior to the set departure/closing date. This will allow Biosafety to consult with the Principal Investigator and perform a walkthrough of the lab to provide recommendations on the most expeditious way to prepare for the move and the final termination of the biohazardous work in the lab. A final Lab Deactivation Inspection will be scheduled accordingly.

Lab Closeout Procedures

- Biosafety cabinets must be decontaminated and the outer surfaces cleaned with a suitable disinfectant; decontamination must be done by a certified professional. Call the MHEC approved vendor to schedule an appointment. The Principal Investigator should present a notice verifying that the decontamination procedure has been completed by the contracted biosafety cabinet certifier.
- Storage freezers should be emptied and the surfaces should be decontaminated with a suitable disinfectant. The former contents must be decontaminated by autoclaving or disposed of in a biohazard bag. Cryostats and liquid nitrogen storage equipment must also be emptied and contents properly disposed of. If the Principal Investigator intends to stay at UMass Amherst but not continue with an IBC approved project, then only the biological agents that were approved for use on the application need to be disposed.
- Account for all specimens stored outside the lab. Specimens stored in a cold room or an incubator in an adjacent tissue culture room should be autoclaved or disposed of in a biohazard bag.
- Medical waste such as used sharps containers or biohazard bags must be disposed of and the storage areas for the medical waste cleaned with a suitable disinfectant.
- Any biohazard labels must be removed from surfaces. The outer surface of all equipment and any work surface must be decontaminated with a suitable disinfectant.
- The Biohazard /Universal Precautions sign must be removed from door.

Disposal of Used Lab Equipment

Used laboratory equipment, such as incubators, refrigerators and freezers, must be thoroughly decontaminated prior to disposal or release to another PI. Laboratory equipment that was used in conjunction with biological research may have residual contamination resulting from chemicals and/or radioactive materials.

- Wear appropriate personal protective equipment. At a minimum wear gloves, lab coat, safety glasses with side shields or goggles and a respirator if chemical vapors/odors are anticipated (contact EH&S for respirator information).
- Remove all specimens and/or laboratory materials.
- Remove all biohazard labels or stickers from the surface of the equipment.
- Be sure that the equipment surface can be safely cleaned with a chemical disinfectant. Make sure that the equipment was not used to store water reactive chemicals, corrosives or strong oxidizers that may incompatibly react during the decontamination process.
- Apply a chemical disinfectant to the surface of the equipment and allow the disinfectant time to inactivate potential contamination.
- Ensure that the surface is rinsed to remove the disinfectant.
- Dispose of PPE properly and wash hands thoroughly.

Do not open internal compartments of equipment for decontamination. If the internal compartments of a piece of equipment are grossly contaminated with biohazardous material, label or tag the equipment as potentially biohazardous. Notify the Biosafety Manager and a decision will be made whether the equipment is safe for disposal.

Place a notice on the equipment stating that you have decontaminated the equipment in accordance with guidelines from Biosafety. Place the date and the initials of the individual that performed the decontamination on the notice. Please record which disinfectant was used on the notice as well.

When the equipment is ready for pick up, place a Surplus Equipment Disposal Request

Appendix A: Biosafety Levels for Biological Agents

Risk Groups: Bacteria

Genus	Species	Biosafety Level	Select Agent
Acinetobacter	lwoffi	2	
Acinetobacter	calceticus	2	
Actinobacillus	actinomycetemcomitans	2	
Actinobacillus	spp	2	
Actinomadura	madurae	2	
Actinomadura	pelletieri	2	
Actinomyces	bovis	2	
Actinomyces	gerencseriae	2	
Actinomyces	israelii	2	
Actinomyces	naeslundii	2	
Actinomyces	pyogenes	2	
Actinomyces	spp	2	
Aerococcus	spp	2	
Aeromonas	hydrophila	2	
Aeromonas	punctata	2	
Amycolata	autotrophica	2	
Arachnia	propionica	2	
Arcanobacterium	haemolyticum	2	
Arizona	hinshawii	2	
Bacillus	anthracis	3	+
Bacillus	cereus	2	
Bacteroides	fragilis	2	
Bacteroides	spp	2	
Bartonella	bacilliformis	3	
Bartonella	elizabethae	3	
Bartonella	spp.	3	
Bartonella	henselae	2	
Bartonella	quintana	2	
Bartonella	vinsonii	2	
Bordetella	spp	2	
Bordetella	bronchiseptica	2	
Bordetella	parapertussis	2	
Bordetella	pertussis	2	
Borrelia	burgdorferi	2	
Borrelia	duttoni	2	
Borrelia	recurrentis	2	
Borrelia	spp	2	
Botulinum neurotoxin			+
Branhamella	catarrhalis	2	
Brucella	abortus	3	+
Brucella	canis	3	
Brucella	melitensis	3	+
Brucella	ovis	3	
Brucella	spp	3	
Brucella	suis	3	+
Burkholderia	mallei	3	+

Genus	Species	Biosafety Level	Select Agent
Burkholderia	pseudomallei	3	+
Calymmatobacterium	granulomatis	2	
Campylobacter	coli	2	
Campylobacter	fetus	2	
Campylobacter	jejuni	2	
Campylobacter	spp	2	
Campylobacter	sputorum	2	
Capnocytophaga	spp	2	
Cardiobacterum	hominis	2	
Chlamydia	pneumoniae	2	
Chlamydia	psittaci	2	
Chlamydia	spp	2	
Chlamydia	trachomatis	3	
Citrobacter	spp	2	
Clostridium	botulinum	3	+
Clostridium	chauvoei	2	
Clostridium	difficile	2	
Clostridium	equi	2	
Clostridium	haemolyticum	2	
Clostridium	histolyticum	2	
Clostridium	novyi	2	
Clostridium	perfringens	2	
Clostridium	septicum	2	
Clostridium	sordelli	2	
Clostridium	tetani	2	
Corynebacterium	bovis	2	
Corynebacterium	diphtheriae	2	
Corynebacterium	equi	2	
Corynebacterium	haemolyticum	2	
Corynebacterium	matruchotii	2	
Corynebacterium	minutissimum	2	
Corynebacterium	pyogenes	2	
Corynebacterium	pseudotuberculosis	2	
Corynebacterium	renale	2	
Corynebacterium	spp	2	
Corynebacterium	ulcerans	2	
Cowdria	ruminantium	3	
Coxiella	burnetii	3	+
Dermatophilus	congolensis	2	
Edwardsiella	tarda	2	
Eikenella	corrodens	2	
Enterobacter	aerogenes/cloacae	2	
Enterobacter	spp.	2	
Enterococcus	spp	2	
Erlichia	spp	2	
Erysipelothrix	rhusiopathiae	2	
Escherichia	coli,enterohemorrhagic	2	

Genus	Species	Biosafety Level	Select Agent
Escherichia	coli, enteroinvasive coli, enteropathogenic coli, enterotoxigenic novocida	2	
Francisella	Tularensis, Type A V	3	+
Francisella	Tularensis, Type B V	2	+
Fusobacterium	necrophorum	2	
Fusobacterium	spp	2	
Gardnerella	vaginalis	2	
Haemophilus	ducreyi	2	
Haemophilus	influenzae	2	
Haemophilus	spp	2	
Helicobacter	pylori	2	
Kingella	kingae	2	
Klebsiella	pneumoniae	2	
Klebsiella	spp	2	
Legionella	pneumophila	2	
Legionella	spp	2	
Leptospira	interrogans	2	
Listeria	ivanovii	2	
Listeria	monocytogenes	2	
Listeria	spp	2	
Mima	polymorpha	2	
Moraxella	spp	2	
Morganella	morganii	2	
Mycobacterium	africanum	3	
Mycobacterium	asiaticum	2	
Mycobacterium	avium-intracelluare	2	
Mycobacterium	bovis	3	
Mycobacterium	chelonei	2	
Mycobacterium	fortuitum	2	
Mycobacterium	kansasii	2	
Mycobacterium	leprae	2	
Mycobacterium	malmoense	2	
Mycobacterium	marinum	2	
Mycobacterium	microti	3	
Mycobacterium	paratuberculosis	2	
Mycobacterium	scrofulaceum	2	
Mycobacterium	simiae	2	
Mycobacterium	szulgai	2	
Mycobacterium	tuberculosis	3	
Mycobacterium	ulcerans	2	
Mycobacterium	xenopi	2	
Mycoplasma	hominis	2	
Mycoplasma	mycoides	3	+
Mycoplasma	pneumoniae	2	
Mycoplasma	spp	2	
Neisseria	gonorrhoeae	2	
Neisseria	meningitidis	2	
Nocardia	asteroides	2	

Genus	Species	Biosafety Level	Select Agent
Nocardia	brasiliensis	2	
Nocardia	farcinica	2	
Nocardia	nova	2	
Nocardia	transvalensis	2	
Nocardia	otitidis-caviarum	2	
Pasteurella	haemolytica	2	
Pasteurella	multocida	3	
Pasteurella	pneumotropica	2	
Peptostreptococcus	anaerobius	2	
Plesiomonas	shigelloides	2	
Porphyromonas	spp	2	
Prevotella	spp	2	
Proteus	mirabilis	2	
Proteus	penneri	2	
Proteus	spp.	2	
Proteus	vulgaris	2	
Providencia	alcalifaciens	2	
Providencia	rettgeri	2	
Providencia	spp	2	
Pseudomonas	aeruginosa	2	
Pseudomonas	mallei	3	
Rhodococcus	equi	2	
Ralstonia	solanacearum	2	
Salmonella	arizonae	2	
Salmonella	cholerasuis	2	
Salmonella	enteritidis	2	
Salmonella	gallinarum-pullorum	2	
Salmonella	meleagridis	2	
Salmonella	paratyphi,A,B,C	2	
Salmonella	typhi	2	
Salmonella	typhimurium	2	
Serpulina	spp	2	
Serratia	marcescens	2	
Serratia	liquefaciens	2	
Shigella	boydii	2	
Shigella	dysenteriae	2	
Shigella	flexneri	2	
Shigella	sonnei	2	
Sphaerophorus	necrophorus	2	
Staphylococcus	aureus	2	
Staphylococcus	epidermidis	2	
Streptobacillus	moniliformis	2	
Streptococcus	agalactiae	2	
Streptococcus	pneumoniae	2	
Streptococcus	pyogenes	2	
Streptococcus	somaliensis	2	
Streptococcus	spp.	2	
Streptococcus	suis	2	
Treponema	carateum	2	

Genus	Species	Biosafety Level	Select Agent
Treponema	pallidum	2	
Treponema	pertenue	2	
Treponema	vincentii	2	
Ureaplasma	urealyticum	2	
Vibrio	cholerae	2	
Vibrio	parahaemolyticus	2	
Vibrio	vulnificus	2	
Xanthomonas	oryzae	2	
Xylella	fastidiosa	3	
Yersinia	enterocolitica	2	
Yersinia	pestis	3	+
Yersinia	pseudotuberculosis	2	

Risk Groups: Rickettsial Agents

BSL – Biosafety Level; SA – Select Agent

Genus	Species	Biosafety Level	Select Agent
Coxiella	burnetii	3	+
Erlichia	sennetsu	2	
Rickettsia	(vole)	2	
Rickettsia	akari	3	
Rickettsia	australis	3	
Rickettsia	canadensis	3	
Rickettsia	conorii	3	
Rickettsia	montanensis	3	
Rickettsia	mooseri	3	
Rickettsia	parkeri	3	
Rickettsia	prowazekii	3	+
Rickettsia	rhipicephali	3	
Rickettsia	rickettsii	3	+
Rickettsia	sennetsu	3	
Rickettsia	sibirica	3	
Rickettsia	spp.	3	
Rickettsia	tsutsugamushi	3	
Rickettsia	typhi (mooseri)	3	
Rochalimaea	quintana	2	
Rochalimaea	vinsonii	2	
Spotted Fever Group - other		3	

Risk Groups: Fungi

Genus	Species	Biosafety Level	Select Agent
Absidia	corymbifera	2	
Aspergillus	flavus	2	
Aspergillus	fumigatus	2	
Aspergillus	spp	2	
Blastomyces	dermatitidis	2/3	
Candida	albicans	2	
Candida	spp	2	
Cladosporium	bantianum	2	
Cladosporium	carrionii	2	
Cladosporium	trichoides	3	
Coccidioides	immitis	3	+
Coccidioides	posadasii	2+	
Cryptococcus	neoformans	2	
Dactylaria	gallopava	2	
Dermatophilus	congolensis	2	
Emmonsia	parva	2	
Epidermophyton	floccosum	2	
Epidermophyton	spp	2	
Exophiala	dermatitidis	2	
Fonsecaea	compacta	2	
Fonsecaea	pedrosoi	2	
Geotrichum	spp	2	
Histoplasma	capsulatum	3	
Histoplasma	farcinimosum	3	
Histoplasma	spp.	3	
Madurella	grisea	2	
Madurella	mycetomatis	2	
Microsporum	spp	2	
Neotestudina	rosatii	2	
Paracoccidioides	brasiliensis	2	
Penicillium	marneffei	2	
Rhizopus	microspous	2	
Sporothrix	schenckii	2	
Trichophyton	rubrum	2	
Trichophyton	spp	2	
Trichosporon	spp	2	
Xylohypha	bantania	3	

Risk Groups: Viruses

Name	Viral Group	Biosafety Level	Select Agent
Absettarov, TBE	Flaviviridae	4	
Acute haemorrhagic conjunctivitis virus (AHC)	Picornaviridae	2	
Adenovirus, human, all types Types 1, 2, 3, 4, 5, 7 Types 40, 41	Adenoviridae	2	
African horse sickness disease	Reoviridae	3	+
African swine fever virus	Adenoviridae	3	+
Aino	X-Arboviruses	2	

Name	Viral Group	Biosafety Level	Select Agent
Akabane	X-Arboviruses	3	+
Alastrim	Poxviridae	4	+
Aleutian Disease Virus	Parvoviridae	2	
Araguari	X-Arboviruses	3	
Astroviridae	Astroviridae	2	
Avian influenza virus	Orthomyxoviridae	3	+
Avian myeloblastosis virus	Retroviridae	2	
Barmah Forest	Togaviridae	2	
Batama	X-Arboviruses	2	
Batken	X-Arboviruses	2	
Bebaru virus	Togaviridae	2	
Bhanja	X-Arboviruses	3	
Bimbo	X-Arboviruses	2	
Bluetongue	X-Arboviruses	3	+
Bobaya	X-Arboviruses	3	
Bobia	X-Arboviruses	2	
Bovine Respiratory Synctial virus	Paramyxoviridae	2	
Bovine Rhinotracheitis	Herpesviridae	2	
Bovine spongiform encephalopathy (BSE)	Unconventional agents, prions	3	
Buenaventura	X-Arboviruses	3	
Bunyavirus	Bunyaviridae	2	
Cabassou	X-Arboviruses	3	
Cache valley	X-Arboviruses	2	
California encephalitis virus	Bunyaviridae	2	
Camel pox virus	Poxviridae	2	
Cardiovirus	Picornaviridae	2	
Central European Tick-borne encephalitis virus, TBE	Flaviviridae	4	
Cercopithecine herpes virus Herpesvirus simiae (B virus, herpes virus)	Herpesviridae	3	
Chikungunya virus	Togaviridae	2/3	
Chim	X-Arboviruses	2	
Classical swine fever virus	Flaviviridae	3	+
Cocal	X-Arboviruses	2	
Congo Crimean haemorrhagic fever TBE	Bunyaviridae	4	+
Coronavirus	Coronaviridae	3 SARS	+
Cowpox virus	Poxviridae	2	
Coxsackie	Picornoviridae	2/3	
Creutzfeldt-Jacob disese	Unconventional agents/prion	2/3	
Cytomegalovirus (CMV) Genus Lymphocryptovirus	Herpesviridae	2	
Dengue virus	Flaviviridae	2	
Dhori	X-Arboviruses	2	
Dugbe	X-Arboviruses	3	
Eastern equine encephalomyelitis (EEE)	Togaviridae	2/3	+
Ebola virus	Filoviridae	4	+
Echoviruses	Picornoviridae	2	
Elephantpox virus (variant of cowpox)	Poxviridae	2	
Encephalomyocarditis virus	Picornavirus	2	

Name	Viral Group	Biosafety Level	Select Agent
Enterovirus	Picornoviridae	2	
Epidemic Diarrhea Infant Mice viruses		2	
Epstein-Barr virus (EBV)	Herpesviridae	2	
Everglade virus	Togaviridae	3	
Far Eastern Tick-borne encephalitis	Flaviviridae	3	+
Feline leukemia virus, FeLV	Retroviridae	2	
Feline sarcoma virus, FeSV	Retroviridae	2	
Flexal (South American hemorrhagic fever virus)	X-Arboviruses	3	+
Foot and Mouth disease virus	Picornaviridae	3	+
Gammaherpes	Herpesviridae	2	
Ganjam	X-Arboviruses	3	
Garba	X-Arboviruses	2	
Germiston	X-Arboviruses	3	
Gerstmann- Straussler- Scheinker syndrome	Unconventional agents, prions	2/3	
Getah	X-Arboviruses	2	
Gibbon leukemia virus (GaLV)	Retroviridae	2	
Goat pox virus	Poxviridae	3-Ag	+
Gordil	X-Arboviruses	2	
Guanarito (South American hemorrhagic fever virus)	Arenaviridae	4	+
Guaratuba	X-Arboviruses	2	
Hantaan (Korean haemorrhagic fever)	Bunyaviridae	3	
Hanzalova,TBE	Flaviviridae	4	
Hart Park virus	Rhabdoviridae	2	
Hazara virus	Bunyaviridae	2	
Hepatitis A virus, human enterovirus type 72	Picornoviridae	2	
Hepatitis B virus	Hepadnaviridae	2	
Hepatitis C virus	Togaviridae	2	
Hepatitis D (Delta) virus (b)	Hepadnaviridae	2	
Hepatitis E virus	Calciviridae	2	
Herpes simplex viruses	Herpesviridae	2	
Herpesvirus ateles	Herpesviridae	2	
Herpesvirus simiae (B virus)	Herpesviridae	4	
Herpesvirus zoster (Varicella)	Herpesviridae	2	
Hog Cholera virus	Flaviviridae	3	
Human B lympho- tropic virus	Herpesviridae	2	
Human Immunodeficiency virus (HIV) Types 1 & 2 Oncornavirus C	Retroviridae	2+	
Human T-cell lymphotropic viruses (HTLV)	Retroviridae	2+	
Hypr,TBE	Flaviviridae	4	
Ibaraki	X-Arboviruses	2	
Influenza virus, Types A-C	Orthomyxoviridae	2	
Inhangapi	X-Arboviruses	2	
Inini	X-Arboviruses	2	
Israel Turkey Mening.	X-Arboviruses	2+	
Issyk-Kul	X-Arboviruses	3	
Itaituba	X-Arboviruses	2	

Name	Viral Group	Biosafety Level	Select Agent
Japanese B encephalitis	Flaviviridae	3	
Japanese encephalitis, Nakayama	Flaviviridae	3	
Junin virus (South American hemorrhagic fever virus)	Arenaviruses	4	+
K (Rate) virus	Papovaviridae	2	
Kairi(x)	X-Arboviruses	2	
Khasan, Koutango	X-Arboviruses	2	
Kokobera	Flaviviridae	2	
Kumlinge,TBE	Flaviviridae	4	
Kunjin	Flaviviridae	2	
Kuru	Unconventional agents/prion	2/3	
Kyasanur Forest, TBE	Flaviviridae	4	+
Kyzylagach	X-Arboviruses	2	
LaCrosse virus	X-Arboviruses	2	
Lactic Dehydrogenase Elevating virus	Arenaviridae	2	
Langat virus	X-Arboviruses	2	
Laryngotracheitis virus	Herpesviridae	2	
Lassa fever virus	Arenaviruses	4	+
Lentiviridae , except HIV-1 and HI	Retroviridae	2+	
Looping ill , TBE	Flaviviridae	3	
Lumpy skin disease virus	Poxviridiae	3	+
Lymphocytic choriomeningitis (neurotropic) virus	Arenaviruses	3	
Lymphocytic choriomeningitis virus	Arenaviruses	2	
Machupo virus (South American hemorrhagic fever virus)	Arenaviruses	4	+
Malignant catarrhal fever	Herpesvirus	3	
Marburg virus	Filoviridae	4	+
Mayaro virus	Togaviridae	2	
Measles virus	Paramyxoviridae	2	
Menangle virus	Paramyxoviridae	3	
Middelburg	X-Arboviruses	2	
Milker's node virus	Poxviridae	2	
Molluscum contagiosum virus	Poxviridae	2	
Monkeypox virus	Poxviridae	3	+
Mopeia virus (other Tacaribe viruses)	Arenaviruses	3	
Morbillivirus,except Rinderpest	Paramyxoviridae	3/4	
Mouse Encephalomyelitis virus	Picornaviridae	2	
Mouse Hepatitis virus	Coronaviridae	2	
Mouse Leukemia virus	Retroviridae	2	
Mucambo virus	Togaviridae	3	
Mumps virus	Paramyxoviridae	2	
Mouse Pneumonia virus	Paramyxoviridae	2	
Murray Valley encephalitis (Australia encephalitis)	Flaviviridae	3	
Myxomatosis virus	Poxviridae	2	
Nairobi Sheep Disease	Bunyaviridae	3	
Nariva, Negishi	X-Arboviruses	2	
Ndumu	Togaviridae	2	
New Minto, Nodamura, Northway	X-Arboviruses	2	

Name	Viral Group	Biosafety Level	Select Agent
Newcastle Disease virus	Paramyxoviridae	2/3	+
Nipah and Hendra complex viruses	Paramyxoviridae	4	+
Norwalk virus	Calciviridae	2	
O'Nyong-Nyong virus	Togaviridae	2	
Omsk (hemorrhagic fever) TBE	Flaviviridae	4	+
Orf virus	Poxviridae	2	
Oropouche virus	Bunyaviridae	3	
Ouango, Oubangui	X-Arboviruses	2	
Papillomaviruses (human)	Papovaviridae	2	
Parainfluenza virus Type 3, SF4 strain	Paramyxoviridae	2	
Parainfluenza viruses	Paramyxoviridae	2	
Paramushir, Piry	X-Arboviruses	2	
Paravaccinia virus	Poxviridae	2	
Parvovirus (human)	Parvoviridae	2	
Peste des petits ruminants	Paramyxoviridae	3	+
Polioviruses	Picornaviridae	2	
Powassan	Flaviviridae	3	
Prospect Hill virus	Bunyaviridae	2	
Pseudorabies virus	Herpesviridae	2	
Puumala virus	Bunyaviridae	3	
Rabbitpox virus (vaccinia variant)	Poxviridae	2	
Rabies virus	Rhabodoviridae	2	
Razdan	X-Arboviruses	2	
Respiratory syncytial virus	Paramyxoviridae	2	
Rhadinovirus, except H.ateles,H. saimiri	Herpesviridae	2	
Rhinovirus	Picornaviridae	2	
Rift Valley Fever, (Zinga virus)	Bunyaviridae	3	+
Rochambeau	X-Arboviruses	2	
Rocio	Flaviviridae	3	
Ross River virus	Togaviridae	2	
Rotavirus (human)	Reoviridae	2	
Rous sarcoma virus	Retroviridae	2	
Rubivirus (Rubella)	Togaviridae	2	
Russian spring- summer encephalitis, TBE	Flaviviridae	4	
Sabia (South American hemorrhagic fever virus)	Arenaviridae	4	+
Sagiyama	X-Arboviruses	2	
Salanga, Santa Rosa, Saumarex Reef	X-Arboviruses	2	
Sammarez Reef	Flaviviridae	2	
Sandfly fever virus	Bunyaviridae	2	
Scrapie	Unconventional agents prions	2	
Semliki Forest virus	Togaviridae	3	
Sendai virus (murine parainfluenza virus type 1)	Paramyxoviridae	2	
Seoul virus	Bunyaviridae	3	
Sepik, Slovakia, Spondweni	X-Arboviruses	2	
Sheep pox virus	Poxviridae	3-Ag	+
Simian immunodeficiency virus	Retroviridae	3	
Simian T-Cell Leukemia Virus	Retroviridae	2	

Name	Viral Group	Biosafety Level	Select Agent
Sin nombre virus	Bunyaviridae	3	
Sindbis virus	Togaviridae	2	
St. Louis encephalitis	Flaviviridae	3	
Subsclerosing pancencephalitis	Paramyxoviridae	2	
Swine vesicular disease virus	Picornaviridae	3	+
Tacaribe complex	Arenaviridae	2 - 4	
Tamdy, Telok Forest, Tiacotalpan	X-Arboviruses	2	
Tanapox	Poxviridae	2	
Tensaw virus	Bunyaviridae	2	
Tick-borne encephalitis complex	Flaviviridae	4	+
Tick-borne orthomyxoviridae,TBE	Orthomyxoviridae	2	
Tonate virus	Togaviridae	3	
Toroviridae	Toroviridae	2	
Toscana virus	Bunyaviridae	2	
Turlock virus	X-Arboviruses	2	
unassigned herpesviruses HHV 7, HHV8	Herpesviridae	2	
Vaccinia virus	Poxviridae	2	
Variola (major and minor) virus	Poxviridae	4	+
Venezuelan equine encephalomyelitis	Togaviridae/ Alphavirus (Grp A Arbovirus)	3	+
Vesicular stomatitis virus	Lab Adapted Strains	2	
Vesicular stomatitis virus	Rhabdoviridae	3	+ exotic
Wesselsbron virus	Flaviviridae	3	
West Nile fever virus	Flaviviridae	3	
Western equine encephalomyelitis	Togaviridae	2/3	
Whitepox (Variola)	Poxviridae	4	
Woolly Monkey Fibrosarcoma virus	Retroviridae	3	
Yabapox virus (Tana and Yaba)	Poxviridae	2	
Yellow fever virus (vaccine strain 17D)	Flaviviridae	2	
Yellow fever virus, wild type	Flaviviridae	3	
Zinga (See Rift Valley Fever)	Bunyaviridae	3	+

Risk Groups: Parasites

Genus	Species	Group	Biosafety Level
Acanthamoeba	castellani	Protozoa	2
Acanthamoeba	spp	Protozoa	2
Anaplasma	spp		2
Ancylostoma	duodenale	Helminth, Nematode	2
Ancylostoma	spp	Helminth, Nematode	2
Ancylstoma	ceylanicum	Helminth, Nematode	2
Angiostrongylus	cantonensis	Helminth, Nematode	2
Angiostrongylus	costaricensis	Helminth, Nematode	2
Angiostrongylus	spp	Helminth, Nematode	2
Ascaris	lumbricoides	Helminth, Nematode	2
Ascaris	spp	Helminth, Nematode	2
Ascaris	suum	Helminth, Nematode	2
Babesia	divergens	Protozoa	2
Babesia	microti	Protozoa	2
Babesia	spp	Protozoa	2

Genus	Species	Group	Biosafety Level
GENUS	SPECIES	GROUP	BSL
Balantidium	coli	Protozoa	2
Brugia	malayi	Helminth, Nematode	2
Brugia	pahangi	Helminth, Nematode	2
Brugia	spp	Helminth, Nematode	2
Brugia	timori	Helminth, Nematode	2
Capillaria	philippinensis	Helminth, Nematode	2
Capillaria	spp	Helminth, Nematode	2
Clonorchis	sinensis	Helminth, Trematode	2
Clonorchis	viverrini	Helminth, Trematode	2
Coccidia	spp	Protozoa	2
Cyclospora	cayetanensis		2
Cryptosporidium	parvum	Protozoa	2
Cryptosporidium	spp	Protozoa	2
Cysticercus	cellulosae	Helminth, Cestode larva	2
Dicrocoelium	spp	Helminths, Trematode	2
Dipetalonema	perstans	Helminth, Nematode	2
Dipetalonema	spp	Helminth, Nematode	2
Dipetalonema	streptocerca	Helminth, Nematode	2
Diphyllobothrium	latum	Helminth, Cestode	2
Diphyllobothrium	spp	Helminth, Cestode	2
Dipylidium	spp	Helminth, Cestoda	2
Dracunculus	medinensis	Helminth, Nematode	2
Echinococcus	granulosis	Helminth, Cestode	2
Echinococcus	multilocularis	Helminth, Cestode	2
Echinococcus	spp	Helminth, Cestode	2
Echinococcus	vogeli	Helminth, Cestode	2
Entamoeba	histolytica	Protozoa	2
Enterobius	spp	Helminth, Nematode	2
Fasciola	gigantica	Helminth, Trematode	2
Fasciola	hepatica	Helminth, Trematode	2
Fasciola	spp	Helminth, Trematode	2
Fasciolopsis	buski	Helminth, Trematode	2
Giardia	lamblia	Protozoa	2
Giardia	spp	Protozoa	2
Haemobartonella	spp		2
Heterophyes	spp	Helminth, trematode	2
Hymenolepis	diminuta	Helminth, Cestode	2
Hymenolepis	nana	Helminth, Cestode	2
Hymenolepis	spp	Helminth, Cestode	2
Leishmania	braziliensis	Protozoa	2
Leishmania	donovani	Protozoa	2
Leishmania	ethiopica	Protozoa	2
Leishmania	major	Protozoa	2
Leishmania	mexicana	Protozoa	2
Leishmania	peruviania	Protozoa	2
Leishmania	spp.	Protozoa	2
Leishmania	tropica	Protozoa	2

Genus	Species	Group	Biosafety Level
Loa	loa	Helminth, Nematode	2
Loa	spp	Helminth, Nematode	2
Mansonella	ozzardi	Helminth, Nematode	2
Mansonella	perstans	Helminth, Nematode	2
Microsporidium	spp.	Protozoa	2
Naegleria	fowleri	Protozoa	2
Naegleria	spp	Protozoa	2
Necator	americanus	Helminth, Nematode	2
Necator	spp	Helminth, Nematode	2
Onchocerca	spp	Helminth, Nematode	2
Onchocerca	volvulus	Helminth, Nematode	2
Opisthorchis	felineus	Helminth, Trematode	2
Opisthorchis	spp	Helminth, Trematode	2
Paragonimus	spp	Helminth, Trematode	2
Paragonimus	westermanii	Helminth, Trematode	2
Plasmodium	cynomologi	Protozoa	2
Plasmodium	falciparum	Protozoa	2
Plasmodium	malariae	Protozoa	2
Plasmodium	simian parasites	Protozoa	2
Plasmodium	spp	Protozoa	2
Plasmodium	vivax	Protozoa	2
Pneumocystis	carinii	Protozoa	2
Sarcocystis	spp	Protozoa	2
Sarcocystis	suihominis	Helminth, Cestode larva	2
Schistosoma	haematobium	Helminth, Trematode	2
Schistosoma	intercalatum	Helminth, Trematode	2
Schistosoma	japonicum	Helminth, Trematode	2
Schistosoma	mansoni	Helminth, Trematode	2
Schistosoma	mekongi	Helminth, Trematode	2
Schistosoma	spp	Helminth, Trematode	2
Strongyloides	spp	Helminth, Nematode	2
Strongyloides	stercoralis	Helminth, Nematode	2
Taenia	saginata	Helminth, Cestode	2
Taenia	solium	Helminth, Cestode	2
Taenia	spp	Helminth, Cestode	2
Toxocara	canis	Helminth, Nematode	2
Toxocara	spp	Helminth, Nematode	2
Toxoplasma	gondii	Protozoa	2
Toxoplasma	spp	Protozoa	2
Trichinella	spiralis	Helminth, Nematode	2
Trichomonas	vaginalis	Protozoa	2
Trichostrongylus	spp	Helminth, Nematode	2
Trichuris	trichiura	Helminth, Nematode	2
Trypanosoma	brucei	Protozoa	2
Trypanosoma	cruzi	Protozoa	2
Trypanosoma	spp	Protozoa	2
Wuchereria	bancroftii	Helminth, Nematode	2
Wuchereria	spp	Helminth, Nematode	2

Appendix B: Select Agents and Toxins

HHS Select Agents and Toxins
Abrin
Bacillus cereus Biovar *anthracis**
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)[1]
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus[3]
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus[3]
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
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV)
Saxitoxin
South American Haemorrhagic Fever viruses: Chapare Guanarito Junin Machupo Sabia
Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
Far Eastern subtype
Siberian subtype
Kyasanur Forest disease virus
Omsk hemorrhagic fever virus
Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

Overlap Select Agents and Toxins
*Bacillus anthracis**
Bacillus anthracis Pasteur *strain*
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**

Overlap Select Agents and Toxins

Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus[1]

USDA Select Agents andToxins

African horse sickness virus
African swine fever virus
Avian influenza virus3
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum[3]
Mycoplasma mycoides[3]
Newcastle disease virus[2,3]
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus

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

Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly Pyrenochaeta glycines)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

**Denotes Tier 1 Agent*

[1] 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 α-MI and α-GI (shown above) as well as α-GIA, Ac1.1a, α-CnIA, α-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.

[2] 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.

[3] 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. 9/10/13

Appendix C: Plant Biosafety

Biosafety Levels for Experiments involving Plants with rDNA/sNAs and microorganisms

Note: the following definition is used for the term "exotic plant pathogen" per NIH Guidelines:

In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V-G, Footnotes and References of Sections I-IV). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research (Section V-M).

Biosafety Level 1- Plants (BL1-P)

BL1-P includes all experiments with recombinant or synthetic nucleic acid molecule-containing plants and plant-associated microorganisms not covered in Section III-E-2-b or other sections of the NIH Guidelines. Examples of such experiments include:

- those involving recombinant or synthetic nucleic acid molecule-modified plants that are not noxious weeds or
- plants that cannot interbreed with noxious weeds in the immediate geographic area, or
- experiments involving whole plants and recombinant or synthetic nucleic acid molecule-modified non-exotic or microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., Rhizobium spp. and Agrobacterium spp.) Section III-E-2-a.

Biosafety Level 2- Plants (BL2-P)

BL-2P includes:

- Experiments involving modification of plants by recombinant or synthetic nucleic acid molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area. Section III-E-2-b-(1).
- Experiments in which the introduced DNA represents the complete genome of a non-exotic infectious agent into plants. Section III-E-2-b-(2).
- Plants associated with recombinant or synthetic nucleic acid molecule-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems. Section III-E-2-b-(3).
- Plants associated with recombinant or synthetic nucleic acid molecule-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems. Section IIIE-2-b-(4).

- Experiments with recombinant or synthetic nucleic acid molecule-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant or synthetic nucleic acid molecule-modified microorganisms associated with them if the recombinant or synthetic nucleic acid molecule-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems. Section III-E-2-b-(5).

Biosafety Level 3- Plants (BL3-P)

BL3-P includes:

- Experiments involving most exotic (see Section V-M, Footnotes and References of Sections I-IV) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant or synthetic nucleic acid molecule techniques are associated with whole plants. Section III-D5-a.
- Experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V-M, Footnotes and References of Sections I-IV) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation in planta. Section III-D-5-b.
- Experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of < 100 nanograms per kilogram body weight fall under Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms Per Kilogram Body Weight, and require NIH/OBA and Institutional Biosafety Committee approval before initiation. Section III-D-5-d.
- Experiments with microbial pathogens of insects or small animals associated with plants if the recombinant or synthetic nucleic acid molecule-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems. Section III-D-5-e.

Biosafety Level 4- Plants (BL4-P)

BL-4P includes:

- Experiments with a small number of readily transmissible exotic (see Section V-M, Footnotes and References of Sections I-IVa) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops

Containment Practices for Biosafety Level 1- 3 Plants

Biosafety Level 1-Plants (BL 1 - P)

Greenhouse Access	Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.
Records	A record shall be kept of experiments currently in progress in the greenhouse facility
Decontamination and Inactivation	Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
Control of Undesired Species and Motile Macroorganisms	A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility
Concurrent Experiments Conducted in the Greenhouse	Experiments involving other organisms that require containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

Biosafety Level 2-Plants (BL 2 - P)

Greenhouse Access	Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress. Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.
Records	A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility and shall be kept of experiments currently in progress in the greenhouse facility. Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/ OBA and other appropriate authorities immediately (if applicable). Reports to the NIH OSP shall be sent to the Office of Science Policy, National Institutes of

	Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; and on the OSP website. Documentation of any such accident shall be prepared and maintained.
Decontamination and Inactivation	Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
Control of Undesired Species and Motile Macroorganisms	A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
Concurrent Experiments Conducted in the Greenhouse	Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.
Other Practices	A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area. Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container. A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms. An autoclave shall be available for the treatment of contaminated greenhouse materials. If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Biosafety Level 3-Plants (BL 3 - P)

Greenhouse Access	Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall

	be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.
Records	A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility and shall be kept of experiments currently in progress in the greenhouse facility. The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable).
Decontamination and Inactivation	All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.
Control of Undesired Species and Motile Macroorganisms	A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws. Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.
Concurrent Experiments Conducted in the Greenhouse	Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.
Other Practices	A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors. Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism. A

greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact. Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms. Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal. Personnel are required to thoroughly wash their hands upon exiting the greenhouse. All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations. An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility. An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse. The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside.

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

01. NIH Guidelines Section V-G. A U.S. Department of Agriculture permit, required for import and interstate transport of plant and animal pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

02. NIH Guidelines Section V-M. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V-G, Footnotes and References of Sections I-IV). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

Appendix D: Arthropod Containment

An ad hoc committee of concerned vector biologists including members of the American Committee Medical Entomology (ACME), a subcommittee of the American Society of Tropical Medicine and Hygiene (ASTMH), and other interested persons drafted the "Arthropod Containment Guidelines." The Arthropod Containment Guidelines (ACG) provide principles of risk assessment for arthropods of public health importance. The risk assessment and practices are designed to be consistent with the NIH Guidelines for recombinant DNA research and BMBL. Arthropods included are those that transmit pathogens; however, those arthropods that cause myiasis, infestation, biting, and stinging are not included. The ACG also specifically exclude most uses of *Drosophila* spp.

The ACG were published in Vector Borne and Zoonotic Diseases. They are freely downloadable from www.liebertonline.com and at the ACME Web site: www.astmh.org.

The ACG recommend biosafety measures specific for arthropods of public health importance considering that:

- Arthropods present unique containment challenges not encountered with microbial pathogens.
- Arthropod containment has not been covered specifically in BMBL or the NIH Guidelines.

The ACG contain two sections of greatest interest to most researchers:

1. The Principles of Risk Assessment that discusses arthropods in the usual context (e.g., those known to contain a pathogenic agent, those with uncertain pathogens, and those with no agent).
2. They also consider the following:
    - Biological containment is a significant factor that reduces the hazards associated with accidental escape of arthropods.
    - Epidemiological context alters the risks of an escape and its impact on the location or site in which the work is performed.
    - The phenotype of the vector, such as insecticide resistance; and
    - Genetically modified arthropods with an emphasis on phenotypic change.

Four Arthropod Containment Levels (ACL 1 – 4) add increasingly stringent measures and are similar to biosafety levels. The most flexible level is ACL-2 that covers most exotic and transgenic arthropods and those infected with pathogens requiring BSL-2 containment. Like BMBL, each level has the following form:

- Standard practices;
- Special practices;

- Equipment (primary barriers);
- Facilities (secondary barriers). The ACG does not reflect a formal endorsement by ACME or ASTMH. The guidelines are subject to change based on further consideration of the requirements for containment of arthropods and vectors.

References

1. American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene. Arthropod containment guidelines. A project of the American Committee of Medical Entomology and American Society of Tropical Medicine and Hygiene. Vector Borne Zoonotic Dis. 2003; 3: 61-98.

Arthropod Containment Guidelines

The Arthropod Containment Guidelines are an important product of the work ACME does to support and advocate for vector biology research nationally and globally. The guidelines provide a reference for research laboratories to assess risk and establish protocols for the safe handling of arthropod vectors of human and animal disease agents. Open Access in Vector Borne and Zoonotic Diseases, February 2019, Vol 19, No. 3 .

Summary of Arthropod Containment Levels (Table 1)

Arthropod containment Level	**1**	**2**	**3**	**4**
Arthropods free of specific pathogens	Indigenous/ no change in local fauna Exotic/ inviable or transient only	Exotic with establishment potential or transgenic	Not at UMass	Not at UMass
Infection Status	Up to BSL-1	Up to BSL-2	Up to BSL-3	Up to BSL-4
Practices	ACL-1 standard handling practices	ACL-2 and BSL-2 limited access, training, signage, containment, and disposal	N/A	N/A
Primary Barriers	Species appropriate containers	Appropriate PPE, escape proof containers	N/A	N/A

Secondary Barriers	BSL-2 facilities, breeding sites, harborage minimized, pest control	N/A	N/A

General guidelines for best laboratory containment practices are shown for vector species of arthropod that are uninfected (shaded area) or infected (below the bold line) according to biosafety and ACLs. Indigenous species are those species whose current range includes the research location. All others are considered exotic. For uninfected arthropods, containment guidelines take into account the consequences of accidental escape from a laboratory, in which the arthropod would be (1) inviable as a result of exposure to unfavorable conditions; (2) transient because conditions vary such that the arthropod would die during typical year climate cycle; or (3) has potential for establishment because escaped arthropods could reasonably be expected to persist through a typical climatic year. Arthropod containment specifics for each BSL should always be reviewed in the context of a laboratory-, vector-, and pathogen-specific risk assessment that is based on consultation between the investigator and the appropriate institutional oversight committee(s) and according to the constraints of the infrastructure available.
Additional restrictions apply for work with arthropods in association with Select Agents.

Principles of Arthropod Risk Assessment

Arthropod risk assessment is primarily a qualitative judgment that cannot be based on a prescribed algorithm. Several factors must be considered in combination: the agents transmitted, whether the arthropod is or may be infected, the mobility and longevity of the arthropod, its reproductive potential, biological containment, and epidemiological factors influencing transmission in the proposed location or region at risk. Arthropod vectors of infectious agents can be assigned to the following discrete categories. Each category has a range of risks that need to be assessed.

Arthropods known to be free of specific pathogens

Risk from these materials to laboratorians is similar to that experienced by the general public: nuisance due to consequences of escape and temporary or permanent establishment. Consequently, the public health risk is likely to be low unless epidemiological conditions exist that could reasonably be expected to result in an increase in transmission of an endemic disease in that particular region, or establishment of the released vector leads to significant risk of future transmission potential for an exotic pathogen. In the event that establishment is likely, the arthropod must be handled under more stringent containment conditions. If an accidental release occurs, followed by even transient establishment of an uninfected arthropod, the probability of increased transmission must be considered in the context of the location in which the work will be performed or in regions to which escaped arthropods could likely migrate. For example, escape of an exotic malaria vector in a malarious region has a significantly higher

probability of increasing transmission and therefore a higher risk than escape in a nonmalarious region. The pathogenicity of the agent and availability of treatments and drugs should also be considered. Answers to the following questions will affect the level of risk due to accidental escape of uninfected arthropods:

- Is the arthropod species already established in the locale?
- If the arthropod is exotic, is it likely that the arthropod would become temporarily or permanently established in the event of accidental escape?
- Does the arthropod have a known or characterized insecticide-resistant genotype or phenotype? Could the arthropod be realistically controlled or locally eradicated by traditional methods (e.g., spraying, trapping) in the event of escape?
- Are the agents that the arthropod is known to transmit cycling in the locale, or has the agent been present in the past?
- Are agents that the arthropod could reasonably be expected to transmit to animals present in the locale?
- Would accidental release of the arthropod significantly increase the risk to humans and animals above that already in existence in the event of introduction of exotic pathogens in the area?
- In the case of zoonotic diseases, does the animal reservoir exist in the locale, and, if so, what is the infection status?
- Was the exotic arthropod derived from a subpopulation (strain, geographically distinct form) whose phenotype is known or suspected to vary in ways that could reasonably be expected to significantly increase its vector competence? If so, it should be handled under the more stringent conditions within ACL-2 (described below) even if uninfected.
- Are disabled strains available, whose viability after escape would be limited (e.g., eye-color mutants, cold sensitive)?

Arthropods known to contain specific pathogens

Arthropods that are known to be, or reasonably suspected of being, infected with infectious agents always have risks that must be identified, and appropriate precautions must be taken for worker and public health safety. The characteristics of most known infectious agents have been well-defined and are the starting point for determining risk from these arthropods. Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies.

The pathogenicity of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease), is the most important consideration in assessing the risk due to accidental exposure to an infected arthropod vector. As the initial criterion, it is clear that the more severe the potentially acquired disease, the higher the risk.

ACL-2 has broad latitude in specific practices. This reflects, in part, the widely differing degrees of effects of arthropod-borne agents, many of which fall within the BSL-2 level. Considerable variation in morbidity and mortality exists within the level 2 classification. Higher containment levels are recommended for agents that cause disease in humans considered potentially severe, life threatening, or cause residual damage. The possible natural and artificial modes of infection (e.g., parenteral, airborne, ingestion) of the agent are considered. This is essential to prevent infections in laboratory staff. The established availability of an effective prophylaxis or therapeutic intervention is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Medical surveillance is encouraged to ensure that the instituted safeguards provide the expected health outcomes. Risk assessment must also include an evaluation of the experience and skill level of at-risk personnel such as laboratorians, maintenance, housekeeping, and animal care personnel. Additional education may be necessary to ensure the safety of persons working at each BSL.

Arthropods containing unknown infectious agents or whose status is uncertain: diagnostic samples

Establish the most appropriate containment level with the limited information available. Some questions that may help in this risk assessment include the following:

- Why is an infectious agent suspected?
- What route of transmission is indicated?
- Are agents that the arthropod transmits transferred horizontally?
- Are there reasons to believe that a novel or unknown agent is present?
- What epidemiologic data are available?
- What is the morbidity or mortality rate associated with the agent?

Bringing field-collected arthropods into a laboratory may be associated with the possibility that personnel who would not otherwise be exposed to any risk because they do not work in field sites might be placed at risk. Researchers working in field sites often handle arthropods of unknown infection status under conditions that do not allow implementation of typical laboratory precautions, but they are in the field and understand that their actions may expose them to associated risks. Answers to the questions above will assist researchers in determining potential risks and reasonable solutions.

Vector arthropods containing recombinant DNA molecules

Employ the principles of risk assessment of vector arthropods that have been genetically modified, typically via recombinant DNA technology. This includes both vector arthropods that contain modified microbes and which themselves are genetically modified. These principles

primarily address the public health significance of the modified organisms rather than environmental concerns.

Among the points to consider in work with recombinant arthropod vectors and those containing recombinant microbes are the following:

- Does the inserted gene encode a product known or likely to alter the vector capacity or competence for pathogens it is known to transmit?
- Does the inserted gene cause phenotypic changes that could significantly affect the ability to control the arthropod if there were an accidental escape, for example, an insecticide resistance marker?
- Does the modification have the potential to alter the range or seasonal abundance of the arthropod? If so, would the new range increase the likelihood that the vector could transmit new pathogens?
- Is the modified strain disabled in a way that viability after escape would be limited (e.g., eye-color mutants, cold-sensitive)?
- Does the modification have the potential to increase the reproductive capacity of the arthropod that carries it?
- Is the phenotype conferred by the modification, including its marker and other expressed genes, if any, consistently expressed after numerous generations of propagation?
- Is the modification undergoing rearrangement or other mutations at a measurable rate?
- Can the DNA transgene vector be mobilized in natural populations?
- Is the host range of the symbiont known?
- Would the modified symbiont pose increased risk to immunocompromised persons relative to the native symbiont?
- Is the entire sequence of the DNA insertion known, and are the coding sequences defined?
- Is horizontal transfer of the transgene to other microbes with which the modified microbe is likely to come into contact possible?
- Is the original insertion site known so that stability can be assessed later?

This list of questions is suggestive and not all-inclusive. The list illustrates the information needed to provide an accurate and conservative assessment of risk to judge the appropriate containment level. Since in many cases the answers to the above questions will not be definitive, it is important that the organization has a properly constituted and informed IBC, as outlined in the NIH Guidelines, to evaluate risk assessment and provide prudent adherence to appropriate safety guidelines for the assigned risk.

Arthropod Containment Levels

Arthropod Containment Level 1

ACL-1 is suitable for work with uninfected arthropod vectors or those infected with a non-pathogen, including (1) arthropods that are already present in the local geographic region regardless of whether there is active vector-borne disease transmission in the locale and (2) exotic arthropods that on escape would be nonviable or become only temporarily established in areas not having active vector-borne disease transmission. This category would include most educational use of arthropod vectors. A summary of the containment levels is provided in Table 1.

Standard practices

Location of arthropods. Furniture and incubators containing arthropods are located in such a way that accidental contact and release are minimized. This may be achieved by locating arthropods out of the flow of general traffic, avoiding hallways, or placing them in closets.

Supply storage.

The area is maintained to allow detection of escaped arthropods. For example, materials unrelated to arthropod rearing and experimentation (e.g., plants, unused containers, clutter) that provide breeding sites and harborages are minimized.

General arthropod elimination.

Accidental sources of arthropods from within the insectary are eliminated. This may be accomplished by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. For example, personnel in mosquito laboratories should immediately eliminate any standing water.

Primary container cleaning and disinfestation.

Practices should be in place such that arthropods do not escape by inadvertent disposal in primary containers. Cages and other culture containers are appropriately cleaned to prevent arthropod survival and escape (e.g., heated to, or chilled below, lethal temperature).

Primary container construction.

Cages used to hold arthropods effectively prevent escape of all stages. Screened mesh, if used, is durable and of a size appropriate to prevent escape. Non-breakable cages are recommended. Bags, rearing trays, and so on effectively prevent leakage and escape.

Disposal of arthropods.

All life stages of arthropods must be killed before disposal. Arthropods may be killed with hot water or freezing before autoclaving or incineration.

Primary container identification and labeling.
Arthropods are identified with descriptive labels to include the species, strain/origin, date of collection, responsible investigator, and so on; labels are firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g., eggs, pupae, hibernating adults) are likewise labeled and (if applicable) housed or stored to prevent progression to, and escape of, a mobile life stage.
Prevention of accidental dispersal on persons or via disposal method.
Personnel take appropriate precautions to prevent transport or dissemination of live mobile arthropods from the insectary by practicing appropriate disposal methods and preventing escapees at every level of containment (primary container, environmental chamber, laboratory, etc.) to prevent dispersal on persons.

Escaped arthropod monitoring.
Investigators assess whether escapes are occurring. An effective arthropod trapping program is recommended to monitor the escape prevention program.

Pest exclusion program.
A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.

Source and harborage reduction.
Harborage and breeding areas are reduced as appropriate. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods.

Notification and signage.
Persons entering the area may be made aware of the presence of arthropod vector species by signage if recommended by an institutional research oversight committee.

Special practices: vertebrate animal use

Institutional approval.
Investigators should consult with their institutional research oversight office if vertebrate animals will be used to feed hematophagous arthropods. The requirement for IACUC and/or IBC review is required.

Housing of vertebrate animals.
Animals used as hosts or blood sources should be housed according to institutional laboratory animal guidelines. If necessary, vertebrate animals may be housed within the

insectary but need to be adequately protected from access by escaped arthropods. Animals not necessary for maintaining arthropods should not be accessible to hematophagous arthropods in the laboratory setting.

Containment during blood feeding.
Special considerations should be taken when hematophagous arthropods are fed on host animals. The primary container must be sufficiently robust to prevent escape during feeding. When handling/removing vertebrate animals after exposure to arthropods, precautions must be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g., concealment in fur, ears, axillae, or other possible hiding places). Finally, all precautions should be taken to prevent arthropods fed on host animals from accidental transfer to host cages and therefore dispersal outside of containment, if animals and their cages are returned to a holding room.

Blood source.
The blood source should be considered a possible source of inadvertent arthropod infection and transmission. Whenever feasible, use of sterile blood or blood from sources known to be specific pathogen free is recommended, whereas use of blood from animals whose disease status is uncertain should be avoided. In some instances, a vector colony is specifically adapted to and will not propagate without human blood acquired directly by feeding on a volunteer. Such arthropods should not be fed a second time on a different volunteer; those fed initially by membrane on animal or human blood should not be allowed to subsequently feed on a human volunteer. The use of human volunteers is strongly discouraged.

Safety equipment (primary barriers)

Gloves.
Latex or nitrile gloves should be used when handling host animals or blood used to feed the arthropods, but local risk assessment and institutional policy may provide exceptions.

Torso apparel.
White laboratory coats, gowns, and/or uniforms should be worn at all times in the insectary when handling blood and vertebrate animals, but local risk assessment and institutional policy may provide exceptions.

Arthropod-specific personal protective equipment.
Personal protective equipment is worn as appropriate, for example, respirators for arthropod-associated allergies, particle masks, and head covers, but local risk assessment and institutional policy may provide exceptions.

Facilities (secondary barriers)

Location of insectary.
The insectary area is separated, if possible, from areas that are used for general traffic within the building.

Insectary doors.
Door openings, whether covered by rigid panels, glass, screens, plastic sheets, or cloth, minimize escape and entrance of arthropods or pests.

Insectary windows.
Windows, if present, effectively prevent escape of the smallest arthropods contained within as well as prevent entry of wild arthropods and pests.

Lack of an insectary.
Arthropods may be maintained at ACL-1 in rooms other than those specifically designed as insectaries. If the facility does not have secondary barriers that would minimize escape or entry of pests, and is not separated from general traffic, specific operating procedures must be developed and tested to mitigate such risks. For example, mosquitoes might be held by a ''cage within a larger cage''; removal of adult mosquitoes accomplished by the aspirator manipulated through cage sleeves placed perpendicular to each other and the sample container loaded entirely within the outer cage. Alternatively, entire mosquito containers may be chilled before aspirating individual mosquitoes. Plexiglas glove boxes might also be used for manipulations, particularly if exotic species are maintained. Nonflying species may be manipulated on designated tables or benches in pans within moats of water, and housed in vials or other containers held within a secondary storage container such as a lidded plastic food container.

Arthropod Containment Level 1

ACL-2 should be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are reasonably suspected of being infected with such agents (diagnostic samples). The PI must perform a risk assessment when deciding whether arthropods are reasonably suspected of being infected with a pathogen. For example, live mosquitoes collected during the course of a disease outbreak and maintained in the laboratory would present more of a risk to laboratory personnel than those that are cold-immobilized or killed before sorting and identifying them for standard surveillance purposes. Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no or only negative effects on viability, survivorship, host range, or vector capacity. **ACL-2 builds on the practices, procedures, containment equipment, and facility requirements of ACL-1.**

It is more stringent in physical containment, disposal, and facility design requirements. Moreover, access is more restricted than ACL-1. The decision to propagate infected exotic arthropods under ACL-2 conditions in active transmission areas or in cases in which establishment is a possibility typically requires that measures that otherwise would only be recommended or preferred must be instituted as policy.

Standard Practices

Location of arthropods.

Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons are unlikely. This may be achieved by locating arthropods in dedicated rooms, closets, incubators located out of the traffic flow, or similar measures. Nonflying arthropods such as ticks are typically held in primary containers (vials) that are placed within an environmentally controlled container such as a desiccator or plastic food container; often, this in turn is held within an environmental chamber.

Supply storage.

The area is designed and maintained to enhance detection of escaped arthropods. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary are located in a designated area and not on open shelves. It is recommended that a closed storage room, cabinets with tight-fitting doors or drawers, be used. Doors and drawers are opened only for access. Insect diet should be kept in sealed containers.

Primary container cleaning and disinfestation.

In addition to cleaning cages and culture containers to prevent arthropod escape as in ACL-1, containers are disinfected chemically and/or autoclaved if used for infected material, according to an IBC-approved protocol and laboratory standard operating procedures.

Primary container construction.

Cages used to hold arthropods are shatter-proof and screened with mesh of a size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during removal and introduction of arthropods are recommended.

Disposal of arthropods.

All life stages of arthropods must be killed before disposal by freezing or other suitable methods. Infected arthropods should be autoclaved, or decontaminated with chemical disinfectants such as 10% bleach or 70% ethanol based on an agent-specific risk assessment. The lack of an autoclave or means of incineration should be evaluated by local risk assessment and appropriate substitutes sought.

Isolation of uninfected arthropods.
Spread of agents to uninfected arthropods is usually a low risk, given that most infections occur via hematophagy. Containers must be clearly marked to easily distinguish infected from uninfected arthropods. It is good practice to separate infected arthropods in a separate room, if possible, to prevent them from being mistaken as being uninfected.

Primary container identification and labeling. As per ACL-1.
Prevention of accidental dispersal via sewer or on persons. Before leaving the insectary and after handling cultures and infected arthropods, personnel wash their hands. Care should be taken to not disperse viable life stages into the drainage system. No infected material is disposed through the sewer. Physical barriers (overlapping sheets and screens) or air curtains are recommended as appropriate; personal protective equipment that is reused (laboratory coats, gowns) should be checked for infestation before exiting the insectary.

Pest exclusion program. As per ACL-1.

Escaped arthropod monitoring.
Investigators assess whether escapes are occurring by instituting an effective arthropod trapping program to monitor the escape prevention program. Oviposition traps, ground-level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes, and so on are recommended. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained. Any evidence of escape should trigger a review of practices and procedures before resuming work.

Source and harborage reduction.
Harborage and breeding areas are eliminated. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods. Equipment in which water is stored or might accumulate (e.g., humidifiers) is screened to prevent arthropod access, or contains chemicals to prevent arthropod survival.

Laboratory sharps.
Disposable sharps should be discarded in puncture-proof containers or as mandated by institutional policy. Forceps, dissecting probes, and other sharps that are reused should be frequently disinfected by chemical disinfection or flame sterilization.

Routine decontamination.
Equipment and work surfaces in the insectary are routinely decontaminated with an effective chemical disinfectant.

Notification and signage.
Persons entering the area should be made aware of the presence of BSL-2 agents in arthropod vectors, but institutions may vary in their policies for security or other reasons. If infected material is present, typically a BSL-2 biohazard sign is posted on the entrance to the insectary, listing all species handled within and is updated whenever new species are introduced or pathogenic infectious agents are present. The lab door card may identify the arthropod species, agent(s) known or suspected to be present, lists the name and telephone number of the responsible person(s), and indicates any special requirements for entering the insectary (e.g., the need for immunizations or respirators).

Procedure design.
All procedures are carefully designed and performed to prevent arthropod escape.

Safety manual.
A site-specific safety manual is to be prepared, approved by the IBC or other institutional review entities, and adopted. The manual contains emergency procedures, standard operating procedures, waste disposal, and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary.

Training.
Laboratory personnel are advised of special hazards and are required to follow instructions on practices and procedures contained in the safety manual. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.

Medical surveillance.
An appropriate medical surveillance program should be considered. All personnel should be educated by the PI about the risks associated with the specific tasks and experiments, as well as the signs and symptoms of any illness caused by the agent(s) under study. Persons who may be at increased risk of acquiring infection, or for whom infection may be unusually hazardous (e.g., immunocompromised), are not allowed in the insectary unless special personal protection procedures are in place to eliminate extra risk.

Access restrictions.
Routine access is limited to trained persons and accompanied guests. Service persons are to be made aware of the hazards present and the consequences of arthropod release and contact with agents that may be present.

Special arthropod handling containers and areas.
Infected arthropods are prevented from release into the laboratory area. A dedicated area for handling infected material is recommended. This is preferably a separate cubicle,

walk-in incubator, or screen room. Additional physical barriers (e.g., glove box, biosafety cabinet) or procedures (incapacitated arthropods, e.g., removing a wing from a mosquito) may be required depending on the risk assessment. BMBL and other sources (Crampton et al. 1997) may provide specific recommendations that can be adopted or modified according to the risk assessment.

Safe transport in the laboratory.
All infectious and potentially infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of arthropods between manipulation and holding areas is in non-breakable secure containers.

Special practices IBC and IACUC approval: as for ACL-1.

Microbial agents classified at BSL-2 require registration with the IBC for review and approval before starting any work. Work with recombinant organisms will need IBC review and approval. IACUC review will also be required for work with vertebrate hosts.

Housing of non-arthropod animals.
Other animals are not accessible to the arthropods. Animals used as hosts or blood sources are not housed with arthropods.

Containment during blood feeding.
Recommendations for ACL-1 containment of arthropods during blood feeding are more stringently assured by special practices and container design, as recommended by the risk assessment.

Blood source: as per ACL-1.
To prevent inadvertent contamination of the clean colony, sources of infection, such as a tube of infected blood, should not be stored in the same refrigerator as a tube of uninfected blood for maintaining uninfected colonies by membrane feeding. Colony arthropods should be maintained in an ACL-1 area and transported to an ACL-2 area for infection; there should be no transport of living arthropods from ACL-2 to ACL-1 without specific risk assessment.

Escaped arthropod handling.
Loose arthropods must be killed and disposed, or recaptured and returned to the container from which they escaped. Infected arthropods must not be killed with bare hands and must be manipulated using filtered mechanical or vacuum aspirators or other appropriate means (e.g., forceps, paintbrushes, gloved hands).

Accidental release reporting.

A release procedure is to be developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in release of infected arthropods from primary containment vessels or that result in overt exposure to infectious material must be reported immediately to the PI who is responsible for ensuring that appropriate and documented action is taken to mitigate the release. The room where the incident occurred is closed off, a warning sign indicating the location, number, and type of material released is prominently posted, and other laboratory personnel are informed until the source is eliminated. Follow-up medical evaluation, surveillance, and treatment are provided as directed by institutional policy and risk assessment, and written records are maintained.

Movement of equipment.

All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.

Safety equipment (primary barriers)

Personal protective equipment should be evaluated as part of the risk assessment. Clothing (primary as well as safety) should conform to institutional policy and to the risk assessment provided to the IBC.

Eye and face protection.

Appropriate face/eye and respiratory protection is worn by all personnel entering the insectary.

Gloves.

Gloves (nitrile) are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.

Torso apparel.

Laboratory coats are worn at all times in the insectary when handling vertebrate animals and infected materials. Universal blood precautions (BMBL5) are recommended when blood is manipulated.

Personal clothing.

Clothing should minimize the area of exposed skin since this can increase the risk of attracting and being bitten by a loose arthropod.

Arthropod-specific personal protective equipment.

Other equipment may be required as determined by the risk assessment. Homogenization of infected arthropods, for example, may require an appropriate respiratory protective device if the procedure is not performed within a biosafety cabinet or glove box.

Facilities (secondary barriers)
An insectary may be a room with a door that may be closed tightly, and it may or may not have environmental controls. Dedicated spaces to be used as insectaries are highly recommended, but resources may not exist to permit such arrangements. The use of infected arthropods may be permitted after risk assessment by the IBC even in the absence of a dedicated space. Ticks, for example, may be safely manipulated within general BSL-2 laboratory settings that are otherwise not considered to be insectaries.

Location of insectary.
The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent passage of the arthropods. Increased levels of physical isolation are recommended, for example, separate buildings, wings, and suites. However, the lack of a dedicated insectary should not imply that infected arthropods may not be manipulated; site specific risk assessments may provide mitigating alternative arrangements. For example, nonflying infected arthropods such as ticks or fleas may be safely manipulated in a dedicated area within a BSL-2 laboratory using a moat system (pan within a pan of water) and accounting for all specimens.

Insectary doors.
Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling arthropod escape. For example, the two contiguous doors must not be opened simultaneously. Internal doors may open outward or be sliding, and are kept closed when arthropods are present. Self-closing doors are highly recommended. Additional barriers (e.g., screened partitions, hanging curtains) may be required by the risk assessment. Alternative arrangements may be specified by risk assessment in the absence of a dedicated insectary.

Insectary windows.
Windows are not recommended, but if present cannot be opened and are well sealed. Windows should be resistant to breakage (e.g., double paned or wire reinforced).

Vacuum systems.
Vacuum devices are appropriately filtered to prevent transfer and exhausting of arthropods.

Interior surfaces.

The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are preferably light colored so that a loose arthropod can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Light-colored floors are also highly recommended, smooth and uncovered. Ceilings are as low as possible to simplify detection and capture of flying insects. Inability to conform to these recommendations may be mitigated by other physical or procedural methods as indicated by the risk assessment. A static glove box with a light-colored interior, for example, may be used to manipulate infected arthropods where the color of walls and floors cannot be easily changed.

Floor drains.

Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate chemical treatment to prevent survival of all arthropod stages (e.g., mosquito larvae).

Plumbing and electrical fixtures.

Internal facility appurtenances (e.g., light fixtures, pipes, ducting) are minimal since these provide hiding places for loose arthropods. Penetrations of walls, floors, and ceilings are minimal and sealed/caulked. Ideally, light fixtures are flush with the ceiling, sealed, and accessed from above.

Heating, ventilation and air conditioning (HVAC).

Ventilation is appropriate for arthropod maintenance, but does not compromise containment of the agent or arthropod. Examples include the following: exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/barriers are installed to prevent escape of arthropods; the direction of airflow in the insectary is inward; a progressively negative pressure gradient is maintained as distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent escape of flying arthropods; and hanging or air curtains are located in vestibules and doorways. Risk assessments may provide site and task-specific alternatives to these recommendations, for example, the use of a static glove box in which infected arthropods are manipulated may provide adequate security if directional airflow is not possible.

Sterilization equipment.

An autoclave is available, conveniently located in rooms containing arthropods within the insectary building. If an autoclave is not available, an appropriate decontamination system or set of practices and procedures are to be recommended by the risk assessment.

Sink.

The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.

Illumination.
Illumination is appropriate for arthropod maintenance, and does not compromise arthropod containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped arthropods are avoided.

Facility compliance monitoring.
The facility should be evaluated annually for compliance to ACL-2. The PI inspects the facility at least annually to ensure that alterations and maintenance have not compromised the containment characteristics. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods is considered.

Arthropod Containment Levels 3 and 4.

UMass Amherst does not have the facilities available to support this level of research.

Appendix E: Zoonotic Fact Sheet

Brucellosis*

Bacteria

Genus Species	Brucella (B. melitensis, B. abortus, B. suis, B. canis)
Host Range	Infected animals (swine, cattle, goats, sheep, dogs)
Transmission	Skin or mucous membrane contact with infected animals, their blood, tissue, and other body fluids
Symptoms	High and protracted (extended) fever. Infection affects bone, heart, gallbladder, kidney, spleen, and causes highly disseminated lesions and abscess
Incubation	1-15 weeks
Fact	Most commonly reported U.S. laboratory-associated bacterial infection in man
Treatment	Antibiotic combination: streptomycina, tetracycline, and sulfonamides

Salmonellosis

Bacteria

Genus Species	Salmonella (S. cholera-suis, S. enteriditis, S. typhymurium, S. typhi)
Host Range	Domestic (dogs, cats, monkeys, rodents, labor-atory rodents, rep-tiles [especially "turtles], chickens and fish) and herd animals" (cattle, chickens, pigs)
Transmission	Direct contact as well as indirect consumption (eggs, food vehicles using eggs, etc.). Human to "human transmission also possible"
Symptoms	Mild gastroenteritiis (diarrhea) to high fever, severe headache, and spleen enlargement. May lead to focal infection in any organ or tissue of the body)
Incubation	6 hours to 3 days
Fact	Fatality rate of 5-10%
Treatment	Antibiotic combination: chloramphenicol, neomycin, ampicillin

Shigellosis*

Bacteria

Genus Species	All Shigella species
Host Range	Captive non-human primates
Transmission	Oral-fecal route
Symptoms	Ranges from asymptomatic carrier to severe bacillary dysentery with high fevers, weakness, severe abdominal cramps, prostration, edema of the "face and neck, and diarrhea with blood, mucous and inflammatory" cells
Incubation	Varies by species. 16 hours to 7 days.
Fact	Highly infective. Low number of organisms capable of causing infec-tion. Rate of "infection in im-ported monkeys can be high"
Treatment	Intravenous fluids and electrolytes, Antibiotics: amoxicillin, "trimethoprin- sulfamethoxazole"

Leptospirosis

Bacteria

Genus Species	Leptospira interrogans
Host Range	Animal, human urine
Transmission	Direct contact with urine of infected dogs, mice or rats. Indirect contact with urine "contaminated materials. Droplet transmission via" aerosols of urine
Symptoms	Phase 1: headache, muscle ache, eye pain with bright lights, chills and fever. Phase 2: fever with stiffness of the neck and inflammation of the nerves to the eyes, brain, spinal column
Incubation	7-12 Days
Fact	Leptospirosis associated with liver and kidney disease is "called Weil's syndrome," characterized by jaundice
Treatment	Doxycycline and penicillin. Severely ill patients may need IV "fluids, antibiotics and dialysis"

Relapsing fever

Bacteria

Genus Species	Borreliae spp. [B. recurrentis (louse- borne), B. hemsii (tick-borne)]
Host Range	Animals
Transmission	Tick-borne, blood transfusions
Symptoms	Fever, headache and muscle pain that lasts 4-10 days and subsides. Afebrile period lasting 5-6 days followed by a recurrence of acute symptoms
Incubation	5-15 days
Fact	Epidemic relapsing fever (transmitted by lice) is more severe than endemic relapsing fever (transmitted by ticks)
Treatment	Tetracyclines, chloramphenicol

Tuberculosis

Bacteria

Genus Species	Mycobacterium tuberculosis
Host Range	Primarily humans, cattle, non-human primates, other animals (rodents)
Transmission	Inhalation of aerosol droplets, contaminated equipment, bites
Symptoms	Ranges from fever and fatigue to chronic pulmonary disease (fatal). Lungs, kidney, vasculature (affects all parts of body)
Incubation	2-5 weeks
Fact	Multidrug-resistant TB (MDR TB) is an infection resistant to at least two first-line anti- TB drugs, isoniazid and rifampicin
Treatment	Isoniazid, rifampin, streptomycin, and ethambutol

Melioidosis*

Bacteria

Genus Species	Burkholderia pseudomallei (formerly Pseudomonas pseudomallei)
Host Range	Equines, especially horses and mules; humans are accidental hosts
Transmission	Transmitted by inhaling dust contaminated by the bacteria and when contaminated soil comes in contact with abraded skin
Symptoms	Cholera-like symptoms (fever, chills, prostration). Skin lesions, swollen lymph glands, abscesses septicemia or pneumonia
Incubation	2-4 days
Fact	Relatively uncommon disease for humans, but when left untreated, has 95% fatality rate
Treatment	Chloramphenicol, doxycycline, sulfisoxazole, or cotrimoxazole. IV chloramphenicol for bacteremia

Tularemia*

Bacteria

Genus Species	Francisella tularensis
Host Range	Isolated from 100 species of wild animals (e.g., rabbits, skunk), 9 domestic mammals, 25 species of birds, frogs, and reptiles
Transmission	Arthropods, direct or indirect contact, ingestion of contaminated meats, inhalation of dust, materials contaminated with urine, feces or tissues, bites and scratches
Symptoms	High fever, chills, headache, focal ulcers, swollen lymph nodes
Incubation	1-10 days
Fact	Bacterium formerly known as Pasteurella tularensis
Treatment	Streptomycin, tetracycline

Herpesvirus

Virus

Genus Species	Herpesvirus Type 1 (fever blister, cold sore) and Type 2 (genital herpes), Herpesvirus hominis, Herpes simiae (Herpes B)
Host Range	Human, non-human primates
Transmission	Produce latent infections in host and frequently shed without overt lesions
Symptoms	Frequently asymptomatic. May have vesicular lesions, neurological or flu- like symptoms
Incubation	5 days to 1 month
Fact	Herpes simiae is 100% fatal if untreated; Herpes Types 1 and 2 are not fatal but cause chronic infection from recurrences
Treatment	Acyclovir or valcyclovir will arrest the virus but will not eliminate virus from the host

Poxvirus*

Virus

Genus Species	Monkeypox, vaccinia, cowpox, buffalopox, cantagalo, and aracatuba viruses
Host Range	Non-human primates, swine, cattle, horses, birds
Transmission	Direct skin contact with lesions on infected animals
Symptoms	Localized lesions, rash, fever, sore throat, malaise, encephalitis
Incubation	Generally: 5-10 days after infection
Fact	Poxviruses are the largest and most complex viruses
Treatment	smallpox vaccine, cidofovir, and vaccinia immune globulin (VIG)

Rabies Virus

Virus

Genus Species	Rhabdoviridae, genus Lyssavirus
Host Range	Natural reservoir: bats. All mammals: wild animals (raccoons, rodents, foxes, etc.) "domestic animals (dogs, cats) and" humans
Transmission	Animal bite, contact with infected saliva or tissue
Symptoms	Headache, fever, malaise, nervousness, dilation of pupils, salivation, excessive perspiration, insomnia, paralysis of throat "muscles, inability to swallow, convulsions, seizures, generalized" paralysis and death
Incubation	3-8 weeks
Fact	Untreated, the fatality rate is 100%; Post- exposure treatment is effective until day 6 post-infection
Treatment	Antirabies vaccine before clinical onset of symptoms; post-exposure treatment "with rabies immune globulin & vaccine"

Viral Hemorrhagic Fever*

Virus

Genus Species	Multiple species: Filoviridae ; Ebola virus, Lassa virus, Marburg virus
Host Range	Humans, non-human primates (Cynomolgous monkeys)
Transmission	Contact with blood and body fluids of infected animals
Symptoms	Severe fever, sore throat, cough, diarrhea, vomiting, hemorrhage and death
Incubation	2-21 days (5-12 days in most cases)
Fact	50-90% fatality rate for Ebola virus; 25% mortality rate for Marburg virus; 15-20% mortality for Lassa fever virus
Treatment	No vaccines; Treatment directed at maintaining renal function, electrolyte "balance and combating hemorrhage and" shock

Arboviral infections*

Virus

Genus Species	Multiple species: Togaviridae, Flaviviridae, Bunyaviridae, Arenaviridae
Host Range	Ticks, insects, infected animals (deer, birds, rodents, etc.)
Transmission	Ticks, insects, blood transfusion
Symptoms	Various: viremia, lymphadenapothy leading to systemic infection. Can involve CNS (encephalitis), skin/bone marrow/blood vessels (hemorrhagic fevers)
Incubation	Mulltiple Ranges; 14-25 days (Avg. 18 days) post infection
Fact	Causes: Rift Valley fever, Denque fever, Yellow fever; Sandfly (Hantavirus) fever; Omsk hemorrhagic fever, and West Nile virus infections
Treatment	No vaccines for most (except yellow fever virus), no known antivirals; supportive treatment only

Viral Hepatitis

Virus

Genus Species	Hepatitis A, B, C, D (delta), E, F, G
Host Range	Humans, non-human primates (chimpanzee, wooly monkey, gorilla, "Celebes ape, some marmosets"
Transmission	Close contact with infected animals or materials
Symptoms	Fever, anorexia, vague abdominal discomfort, nausea and vomiting, sometimes arthralgias and rash, "often progressing to jaundice; fever may be absent or mild"
Incubation	3-6 weeks
Fact	Hepatitis A has no carrier state; Hepatitis B 20% chronic; "Hepatitis C 85% chronic"
Treatment	Vaccines for Hepatitis A and B only. Treatment with alpha "inter-feron and intra-venous immuno-" globulins (HBIG)

Lymphocytic Choriomeningitis (LCM)

Virus

Genus Species	Multiple arenaviruses
Host Range	Rodents (hamsters, mice, guinea pigs), monkeys and humans
Transmission	Infected mice excrete virus in saliva, urine and feces; man infected through inhalation of aerosolized particles of (urine, feces or saliva) contaminated with virus
Symptoms	Biphasic febrile illness, mild influenza like illness or occasionally meningeal or meningoencephalomyelitic symptoms, transverse myelitis
Incubation	15-21 days
Fact	46 documented laboratory-acquired cases with 5 deaths; cases also reported arising from contaminated cell lines
Treatment	No specific treatment; anti-inflammatory drugs may be useful; No known vaccines

Vesicular Stomatitis*

Virus

Genus Species	Multiple strains of Vesicular Stomatitis Virus (VSV) Rhabdoviridiae
Host Range	Bovine, equine, porcine animals.
Transmission	Probably arthropod-borne via the bite of an infected sandfly, mosquito or blackfly; by direct contact with infected animals (vesicular fluid, saliva)
Symptoms	Infuenza-like illness, malaise, fever, headache, nausea and vomiting
Incubation	24-48 hours
Fact	Documented hazard to personnel (45 laboratory-acquired infections before 1980) handling infected livestock, tissues and virulent isolates
Treatment	Virus is self-limiting and illness is short in duration. (3-6 days)

Sub-viral Agents and Related Diseases (i.e., Scrapie)*

non-RNA/DNA Infectious Protein Virus- like particle

Genus Species	Transmissable Spongiform Encephalopathies (TSE): BSE and vCJD (vCreutzfeld- Jacob Disease)
Host Range	Adult sheep goats, and cows can infect humans
Transmission	Ingeston or handling of brain tissue or unfixed brain cells from infected animals
Symptoms	Degeneration of the nervous system, severe variable alteration of the grey matter of the brain
Incubation	2-5 years
Fact	The agent responsible for TSE's is smaller than the smallest known virus and has not been completely characterized
Treatment	There are no known treatments or vaccines for these TSE's

Amoebic Dysentery

Parasite (protozoa)

Genus Species	Entamoeba histolytica
Host Range	Monkeys can readily transmit the agent to humans
Transmission	Food, water, fomites, insects. Fecal-oral route. Cyst is resistant to drying
Symptoms	Frequent passage of feces/stool, loose stools and vomiting. Variations depending on parasites. Can be frequent urge with high or low volume of stool, with or without some associated mucus and even blood
Incubation	2 days to several months to even years
Fact	Harmless amoebas can live in the intestines for years without causing symptoms. Attacks can last from a few days to weeks
Treatment	Antiamebic drugs (Iodoquinol, metronidazole) and antibiotics to treat associated bacterial infections

Giardiasis

Giardiasis

Genus Species	Giardia lamblia
Host Range	Dogs, monkeys
Transmission	Drinking contaminated water, person-to-person "contact, eating contaminated food, and" direct contact with infected animals
Symptoms	Ranges from asymptomatic to nausea, fatigue, anorexia, severe diarrhea and high fever
Incubation	3-25 days
Fact	Most common waterborne diarrheal disease in humans
Treatment	Quinacrine hydrochloride, "metronidazole, tinidazole," albendazole and furazolidone

Balantadidiasis

Parasite (protozoa)

Genus Species	Balantidium coli
Host Range	Monkeys, pigs, and other nonhuman primates readily transmitted to humans
Transmission	Direct contact with feces, person-to-person transmission
Symptoms	Ranges from asymptomatic to severe diarrhea
Incubation	4-5 days
Fact	Cysts survive for long periods in the environment
Treatment	Tetracycline, Iodoquinol, metronidazole

Malaria

Parasite (protozoa)

Genus Species	Plasmodium species: P. falciparum "P. vivax P. ovale P. malariae"
Host Range	Anopheles mosquito
Transmission	Mosquito bite
Symptoms	Fever, chills sweating, headache, nausea, vomiting, muscle pain, anemia, bloody stools, jaundice, convulsion, coma
Incubation	10 days to 4 weeks after infection; symptoms then cycle every 48 days
Fact	A malaria vaccine has been developed and is being tested in Africa. Results are promising
Treatment	Chloroquine, primaquine phosphate, Malorone

Toxoplasmosis

Parasite (protozoa)

Genus Species	Toxoplasma gondii
Host Range	Amazing lack of host specificity. Primates, "carnivores (felines), rodents, birds, undulates"
Transmission	Consuming under-cooked infected meats; ingestion "of oocysts in milk, food or water; inhalation of oocysts;-contact with soil" containing contaminated cat feces;
Symptoms	Localized lymphadenopathy accompanied with fever, sore throat, rash, pneumonitis, myocarditis, and encephalitis
Incubation	10-23 days following "ingestion of contamin-ated meats, or" inhalation of aerosols
Fact	Affects one third of the human race. "Especially infective to immunosupressed individuals"
Treatment	Sulfonomides (sulfadiazene, "sulfamerazine, sulfamethazine), pyrimethamine"

Ascariasis (Roundworm)

Nematode

Genus Species	Multiple Ascaris species (A. lumbricoides, A. suum)
Host Range	Pigs; Humans are the definitive host
Transmission	Ingestion of contaminated food or water
Symptoms	Lung damage, intestinal symptoms
Incubation	4 to 8 weeks
Fact	Ascaris lumbricoidesis the "largest and, globally, the most widespread of all human intestinal" roundworms
Treatment	Pyrantel pamoate, mebendazole, surgery for removal in lung tissue

Visceral Larval Migrans (VLM)

Nematode

Genus Species	Nematodes of the Toxocara genus (T. canis, T. felis)
Host Range	Dogs, cats
Transmission	Ingestion of eggs through direct contact with feces or contaminated materials
Symptoms	Fever, cough, wheezing, itching/irritation associated with migration of nematodes into tissues. Ocular migration may cause blindness
Incubation	4 to 7 weeks
Fact	More than 80% of all puppies in the U.S. are infected with this nematode
Treatment	Usually a self-limiting disease--treatment only given in severe cases (glucocorticoids and bronchodilators for pulmonary disease)

Strongyloidiasis

Nematode

Genus Species	Strongyloides stercoralis
Host Range	Dogs, cats, monkeys
Transmission	Careless handling of contaminated fecal materials
Symptoms	Abdominal pain, diarrhea, and rash. Less commonly, nausea, vomiting, weight loss and cough. Severe infection can cause severe tissue damage, systemic damage of various tissues in the body and potential death
Incubation	skin 7 hours; lung 1 week; intestines 2 wks; average 4-21 days
Fact	The parasite penetrates the skin and migrates to the lungs. Then it travels up to the mouth and is swallowed into the intestinal tract
Treatment	Ivermectin with Albendazole as the alternative

Trichinosis

Nematode

Genus Species	Trichinella spiralis
Host Range	Generally pigs or cattle
Transmission	Eating undercooked flesh of animals infected with the larvae
Symptoms	Nausea, vomiting, diarrhea, fever, neurological disorders, possible cardiac involvement
Incubation	Abdominal symptoms: 1- 2 days. "Further symptoms 2-8" weeks after infection
Fact	Over 100 species of animals may be a host of this parasite
Treatment	Thiabendazole (Mintezol), Albendazole "(Albenza), Mebendazole" (Vermox), Prednisone

Appendix F: Glossary

Aerosol Transmissible Disease (ATD)
A disease or pathogen for which droplet or airborne precautions are required.

Aerosol Transmissible Disease Plan (ATD plan)
Laboratories should adopt standard biosafety practices to protect lab workers handling materials containing pathogens that may spread through aerosols and cause serious disease.

Biosafety
The application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated.

Biosafety Cabinet (BSC)
An enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens requiring a defined biosafety level. The biosafety cabinets are designed to provide three types of protection:

- Personal protection for the staff from material inside the cabinet
- Protection for the material inside of the cabinet from outside contamination
- Protection for the environment from the material inside of the cabinet

There are three types of BSCs: Class I, II, and III. The use of Class I BSCs is not advised at UMass Amherst. Contact Biosafety if you feel you need to purchase one.

Biosafety Level (BSL)
A set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. The levels of containment range from BSL - 1 to BSL - 4.

Biohazardous Agents
A bacterium, virus, or other microorganism that can cause disease in healthy individuals, animals or plants.

Bloodborne Pathogens (BBP)
Pathogenic microorganisms that are present in human blood and other potentially infectious material (OPIM) and can cause disease.

Bloodborne Pathogens Exposure Control Plan (ECP)
The Bloodborne Pathogen Exposure Control Plan (ECP) helps the Principal Investigator (PI)/ supervisor complete requirements for the Bloodborne Pathogen (BBP) Standard. The PI/supervisor reviews the ECP with input from employees covered by the BBP Standard, with

the goal to minimize personnel exposure to BBPs in blood or other potentially infectious materials (OPIMs).

Engineering Controls

Safety equipment (primary barriers) includes biological safety cabinets, enclosed containers and other designed controls designed to remove or minimize exposures to hazardous biological agents.

Gene Transfer

Delivery of exogenous genetic material (DNA or RNA) to somatic cells for the purpose of modifying those cells.

Institutional Biosafety Committee (IBC)

The committee assures the institution's compliance with federal, state and local regulation of research and teaching activities by reviewing those activities which involve the use of human subjects, laboratory animals, biohazardous agents, and recombinant DNA/synthetic DNA.

NIH Guidelines

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) detail safety practices and containment procedures for basic and clinical research involving recombinant or synthetic nucleic acid molecules, including the creation and use of organisms and viruses containing recombinant or synthetic nucleic acid molecules.

Pathogen

A bacterium, virus, or other microorganism that can cause disease.

Personal Protective Equipment (PPE)

Refers to protective clothing (lab coats, gowns, gloves, etc.) eye protection (safety glasses, goggles, face shields, etc.) or equipment (Biosafety Cabinets) designed to protect the wearer's body from injury or infection.

Recombinant DNA (rDNA)

Refers to DNA which has been altered by joining genetic material from two different sources. It usually involves putting a gene from one organism into the genome of a different organism, generally of a different species.

Synthetic nucleic acid (sNA)

Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e. synthetic nucleic acids).

Training

- Biosafety Training for Laboratory Personnel
- Bloodborne Pathogens Training
- BSL-3 Biosafety Training
- Biosafety Information Sessions by Request

Transgene

A gene that is taken from the genome of one organism and introduced into the genome of another organism by artificial techniques.

Transgenic

An organism that contains genetic material into which DNA from an unrelated organism has been artificially introduced.

Universal precautions

An approach to infection control to treat all human blood and certain human body fluids as if they were known to be infectious for HIV, HBV and other bloodborne pathogens. Universal Precautions includes frequent handwashing, no mouth pipetting, no food or drink in the lab and proper disposal of biohazardous/medical waste, as well as the use of engineering controls and Personal Protective Equipment (PPE). Engineering controls include items such as biosafety cabinets, ventilation systems, closed top centrifuge rotors, etc.; these are the primary methods to control exposure. PPE such as gloves, lab coats, eye protection, face shields or others must be selected and used as appropriate

Viral Vector

Viruses that are used to deliver genetic material into cells.

Appendix G: Contingency Plans and Emergency Procedures

Every laboratory that works with infective microorganisms should institute safety precautions appropriate to the hazard of the organisms and the animals being handled.

Contingency plan

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake and explosion
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people and animals from the premises
5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Epidemiological investigation
9. Post-incident continuation of operations.

In the development of this plan the following items should be considered for inclusion:

1. Identification of high-risk organisms
2. Location of high-risk areas, e.g. laboratories, storage areas, animal facilities
3. Identification of at-risk personnel and populations
4. Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, and fire and police services
5. Lists of treatment and isolation facilities that can receive exposed or infected persons
6. Transport of exposed or infected persons
7. Lists of sources of immune serum, vaccines, drugs, special equipment and supplies
8. Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

Emergency procedures for microbiological laboratories

Puncture wounds, cuts and abrasions
The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention as necessary. The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

Ingestion of potentially infectious material
Protective clothing should be removed and medical attention sought. Identification of the material ingested and circumstances of the incident should be reported, and appropriate and complete medical records kept.

Potentially infectious aerosol release (outside a biological safety cabinet)

All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. The laboratory supervisor and the biosafety office (~30 minutes), to allow aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed (e.g. for 24 h).

Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the biosafety officer. Appropriate protective clothing and respiratory protection should be worn.

Broken containers and spilled infectious substances
Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels. Disinfectant should then be poured over these and left for the appropriate amount of time. The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be swabbed with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant. Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container. Gloves should be worn for all these procedures.

If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets
If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow settling. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 min). In both instances, the biosafety officer should be informed.

Strong (e.g. thick rubber) gloves, covered if necessary with suitable disposable gloves, should be worn for all subsequent operations. Forceps, or cotton held in the forceps, should be used to retrieve glass debris.

All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant known to be active against the organisms concerned. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.

The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

Breakage of tubes inside sealable buckets (safety cups)
All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.

Fire and natural disasters

Fire and other services should be involved in the development of emergency preparedness plans. They should be told in advance which rooms contain potentially infectious materials. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.

After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Infectious materials should be collected in leak-proof boxes or strong disposable bags.

Salvage or final disposal should be determined by biosafety staff on the basis of local ordinances.

Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed in the facility:

1. The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
2. Director of the institution or laboratory
3. Laboratory supervisor
4. Biosafety officer
5. Fire services
6. Hospitals/ambulance services/medical staff (names of individual clinics, departments, and/or medical staff, if possible)
7. Police
8. Medical officer
9. Responsible technician
10. Water, gas and electricity services

Emergency equipment

The following emergency equipment must be available:

1. First-aid kit, including universal and special antidotes
2. Appropriate fire extinguishers, fire blankets

The following are also suggested but may be varied according to local circumstances:

1. Full protective clothing (one-piece coveralls, gloves and head covering – for incidents involving microorganisms in Risk Group 3)
2. Full-face respirators with appropriate chemical and particulate filter canisters
3. Room disinfection apparatus, e.g. sprays and vaporizers
4. Stretcher
5. Tools, e.g. hammers, axes, spanners, screwdrivers, ladders, ropes
6. Hazard area demarcation equipment and notices.

Appendix H: Risk Assessment Template

University of Massachusetts Amherst Environmental Health and Safety

Risk Assessment for ***Biological agent*** use in the Dr. **XXXXX** Laboratory **Locations**

The tables below are used to assess the risk level for each hazard that is identified.

Hazard Likelihood	Description of Likelihood
1. Rare	Will only occur in exceptional circumstances
2. Unlikely	Not likely to occur within the foreseeable future
3. Possible	May occur within the foreseeable future, sporadic exposure is possible
4. Likely	Likely to occur within the foreseeable future, routine exposure is likely
5. Highly Likely	Almost certain to occur within the foreseeable future, consistent exposure is highly likely

Hazard Consequence	Description of Consequence
1. Insignificant	No treatment required
2. Minor	Minor injury requiring First Aid treatment (e.g. minor cuts, bruises, bumps)
3. Moderate	Injury requiring medical treatment or lost time
4. Major	Serious injury (injuries) requiring specialist medical treatment or hospitalization
5. Critical	Loss of life, permanent disability or multiple serious injuries

Risk Assessment Matrix		Hazard Consequence				
		Insignificant	Minor	Moderate	Major	Critical
Hazard Likelihood	Highly likely	Medium	Medium	High	Extreme	Extreme
	Likely	Low	Medium	High	High	Extreme
	Possible	Low	Medium	High	High	High
	Unlikely	Low	Low	Medium	Medium	High
	Rare	Low	Low	Low	Medium	Medium

Assessed Risk Level		Description of Risk Level	Actions
☐	Low	If an incident were to occur, there would be little likelihood that an injury would result.	Undertake the activity with the existing controls in place.
☐	Medium	If an incident were to occur, there would be some chance that an injury requiring First Aid would result.	Additional controls are advised.
☐	High	If an incident were to occur, it would be likely that an injury requiring medical treatment would result.	Control will need to be in place before the activity is undertaken.
☐	Extreme	If an incident were to occur, it would be likely that a permanent, debilitating injury or death would result.	Consider alternatives to doing the activity. Significant control measures will need to be implemented to ensure safety.

Identify risks in the following chart: *(Modify the chart as needed)*

TASK	HAZARD	CONTROL MEASURES	RISK LEVEL	HOW TO IMPLEMENT	RESPONSIBLE PERSON
Agent		BSL-2 laboratory practices		BSL-2 facilities, engineering controls, and administrative controls; PPE; Fact sheet available	
Agent in cells?		ABSL-2 containment practices		ABSL-2 facilities, engineering control, and administrative controls; PPE	
Pipetting or syringe transfer of cells	Production of aerosols	Perform in biosafety cabinet; use pipet filters		BSC available, Training, SOP's; demonstration; PPE	
Centrifugation	Production of aerosols	Use of sealed rotors or caps		Sealed rotors and caps must be available for use; PPE	
Vortexing	Production of aerosols	Perform in biosafety cabinet;		BSC available, Training, SOP's; demonstration; PPE	
Splash/spray	Production of aerosols	Use threaded microfuge tubes		BSC available, Training, SOP's; demonstration; PPE	
Small volume spills	Production of aerosols	Spill procedures in place		Training, SOP's for spills; demonstration; PPE	
Opening tubes/vials	Production of aerosols	Vacuum tubes opened within BSC		Training, SOP's; demonstration; PPE	
Sharps use: scissors, forceps, needles	Auto-inoculation, cuts	Sharps precautions: no cutting of needle, no re-capping, prompt disposal in approved container		Training, SOP's; demonstration; PPE	
Specimen transport	Loss of containment	Secondary containment: box with lid that has latches		Training, SOP's; demonstration	
Disinfection	Inadequate "kill" of biologicals	Use of freshly prepared 10% bleach solution with contact time of 10 minutes		Training, SOP's; demonstration; PPE	
Harvesting tissues: XXXX	Exposure risk; sharps injury risk	BSL-2 laboratory practices; Perform in biosafety cabinet		BSC available, Training, SOP's; demonstration; PPE	
Spleen processing	Exposure risk; crushing procedure may yield aerosols	BSL-2 laboratory practices; Perform in biosafety cabinet		BSC available, Training, SOP's; demonstration; PPE	
Cell filtration	Production of aerosols	BSL-2 laboratory practices; Perform in biosafety cabinet		BSC available, Training, SOP's; demonstration; PPE	
Cell collection	Production of aerosols	BSL-2 laboratory practices; Perform in biosafety cabinet		BSC available, Training, SOP's; demonstration; PPE	
Facilities	Contaminated work surfaces	Readily disinfected; hand washing area		BSL-2 approved work area	
Waste handling	Production of aerosols; release to environment	Follow UMass Waste Program; Autoclaving		Training, SOP's; demonstration; PPE	
Changing Animal Bedding	Production of aerosols	ABSL-2 containment practices		Training, SOP's; demonstration; PPE	
Animal inoculation	Production of aerosols; sharps injury	Sharps precautions: no cutting of needle, no re-capping, prompt disposal in approved container		Training, SOP's; demonstration; PPE	
Animal handling	Production of aerosols; bite or scratch	ABSL-2 containment practices		Training, SOP's; demonstration; PPE	

A new risk assessment should be performed: before analysis begins, whenever the procedure location is moved, when laboratories are renovated, when new employees begin working, when reagents are changed (manufacturer, type of reagent, etc.), before new equipment is used, and repeated when changes are made to the procedure, facility, and the employees.

Conclusions and Recommendations: (modify the text that is in red to your findings)

- The assessed risk level has been determined to be: **XXXX**
- The risk is **XXXXXX** and will be managed by well-established, routine processes and procedures.
- Vaccination is **not** recommended as there is **no** vaccine available.
- **Human infection is unlikely as the agent is not human infective.** Proper containment must be maintained to avoid animal infectivity within the vivarium. The arthropod vector **(XXXX)** is **not** endemic to our region, therefore an accidental environment release is not likely to have any consequences.
- Standard **BSL-2 (ABSL-2)** containment practices **are sufficient** to address the agent and procedural risks.
- This entire risk assessment should be discussed with and available to all staff that may come into contact with the infected cells, infected animals, and any waste products of this research.

This risk assessment was prepared by:

Signature:

Date:

Forward a copy of this risk assessment to the Biosafety team for review prior to commencing procedures.

Appendix I: Contact Info

Biosafety Staff

Judy LaDuc RBP (ABSA)
Biosafety Officer
Environmental Health and Safety
UMass Amherst
40 Campus Center Way
Draper Hall Room 117
Amherst, MA 01003
Work Phone: 413-545-7293
Cell Phone: 413-687-5476
E-mail: jladuc@ehs.umass.edu
www.ehs.umass.edu

Vacant
Associate Biological Safety Officer
Environmental Health & Safety
UMass Amherst
40 Campus Center Way
Draper Hall Room 117
Amherst, MA 01003
413-545-9846 (office)
413-345-0799 (mobile)
Email:
www.ehs.umass.edu

Emergency Contacts

In case of emergency:
911 or UMPD **413-545-3111**

University Health Services
413-577-5000

EH&S
413-545-2682

Resources:

1. American Biological Safety Association (ABSA) https://absa.org/
2. ABSA Risk groups Tool https://my.absa.org/tiki-index.php?page=Riskgroups
3. Arthropod Containment Levels
   American Committee on Medical Entomology (ACME)
   https://www.liebertpub.com/doi/10.1089/vbz.2018.2431
   www.astmh.org
4. Biosafety in Microbiological and Biomedical Laboratories 5th (BMBL)
   https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF
5. Biosafety Training https://ehs.umass.edu/biological-safety-training-4
6. Biosafety Website https://ehs.umass.edu/biosafety
7. CDC Imports FAQ https://www.cdc.gov/phpr/ipp/faq.htm
8. CDC Permits www.cdc.gov/od/eaipp/
9. Dual Use https://www.phe.gov/s3/dualuse/Documents/P3CO-FinalGuidanceStatement.pdf
10. Environmental Health & Safety Website http://ehs.umass.edu/
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