UCONN

BIOLOGICAL SAFETY MANUAL

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BIOLOGICAL SAFETY MANUAL

1 PURPOSE

To protect employees, students, and others from exposure to biological agents, prevent environmental contamination, comply with applicable federal, state, and institutional requirements, and provide assistance in the evaluation, assessment, and control of biological agents and toxins, recombinant or synthetic nucleic acid molecules, and other biological materials identified or used in clinical trials, research, and teaching.

2 APPLIES TO

The Biological Safety Manual applies to employees, students, and others working in UConn laboratories with biological agents at the Storrs and regional campuses, excluding UConn Health.

3 POLICY STATEMENT

The Biological Safety Manual incorporates guidance from the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) by the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH), applicable federal and state regulations, and University-specific policies and procedures governing the use of biological materials. All UConn workforce members and visitors working in laboratories with biological materials are required to comply with the requirements in the manual.

4 ENFORCEMENT

Violations of this policy and any related procedures may result in appropriate disciplinary measures in accordance with University Laws and By-Laws, General Rules of Conduct for All University Employees, applicable collective bargaining agreements, and the University of Connecticut Student Conduct Code.

5 RESPONSIBILITIES

All individuals must carry out their responsibilities and comply with the requirements in the Biological Safety Manual. The responsibilities of each group are listed below:

5.1 Office of the Vice President for Research (OVPR)

- Provides oversight of research and other sponsored activities through Research Integrity and Compliance (RICS) to ensure compliance with federal, state, and institutional regulations and policies.
- Provides support and training to faculty, students, and staff.
- Works with faculty oversight committees (e.g., Institutional Review Board (IRB), Stem Cell Research Oversight Committee (SCRO), Institutional Animal Care and Use Committee (IACUC), etc.) to promote the ethical and responsible conduct of research and ensure regulatory compliance.

5.2 Institutional Biosafety Committee (IBC)

- Ensures individuals register experiments for research or teaching with the IBC that involve the use of recombinant or synthetic nucleic acid molecule technology and/or biohazard materials as described in this BSM and the IBC Policy and Procedure.
- Reviews and approves the use of recombinant and synthetic nucleic acid molecules (rsNA) and biological materials for research and teaching as defined in the BSM and the IBC Policy and Procedure.
- Ensures UConn employees and students involved in biological research follow the safety guidelines and requirements of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines) and the CDC NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

5.3 Department Head

- Provides leadership to support biosafety research, teaching, and compliance.
- Ensures departmental personnel comply with biosafety program requirements.
- Communicates changes in biosafety policies and procedures to departmental personnel.
- Ensures departmental personnel comply with EHS and University training requirements.
- Enforces safe work practices during the handling and disposal of biohazardous materials.

5.4 Principal Investigator (PI)

The role of Principal Investigator (PI) may be carried out by a faculty member, laboratory director, laboratory manager, course instructor, or other individual in a supervisory role overseeing biological materials. PIs are responsible for the safety and compliance of all individuals working with biological agents in their laboratories. PIs responsibilities are listed below:

- Ensures that all work is conducted in compliance with applicable NIH, CDC, OSHA, and other applicable regulations, guidelines, and standards, as indicated in the BSM.
- Completes a registration for all biological materials and agents in their laboratory.
- Provides an annual update of their registration.
- Prepares a risk assessment for all biological materials (See Risk Assessment).
- Implements the guidelines, policies, and procedures outlined in the BSM.
- Institutes appropriate safe work practices.
- Maintains an up-to date inventory of microorganisms, cells, cell lines, tissue samples, toxins and any other regulated biological materials.
- Provides and ensures staff wear appropriate personal protective equipment.
- Instructs students and staff, on a continual basis, of actual and potential hazards and hazard controls.
- Ensures laboratory personnel (students and staff) complete required safety training(s).
- Ensures lab personnel properly collect, label, and manage biological wastes.
- Reports accidents, injuries, and exposures involving biological agents. Completes the DAS-WC207 First Report of Injury Form to document injuries.
- Ensures that minors (individuals under 18 years old) are continually supervised by qualified laboratory personnel.

5.5 Biosafety Officer

The Biosafety Officer (BSO) is responsible for the oversight and administration of the Biosafety program. The BSO responsibilities include:

- Provides consultation on biological agent risk assessments (See Risk Assessment).
- Recommends risk control measures including personal protective equipment (PPE).
- Consults on issues concerning biosecurity.
- Implements policy guidelines recommended by the IBC.
- Determines streams for biomedical waste disposal.
- Provides training on various biosafety topics.
- Maintains and updates a database of work areas with biological hazards on a continual basis.
- Performs laboratory audits to assure compliance with applicable biosafety requirements.
- Communicates with federal and state regulators to ensure regulatory compliance.
- Works with the IBC regarding research and or teaching projects that use rsNA technology or biological agents that require special and or increased biological containment practices.

- Identifies potential biosafety issues through biosafety audits and interactions with PIs, presents the issues to the IBC, and makes recommendations to address the safety and/or compliance concerns.
- Investigates laboratory accidents and injuries and provides corrective actions to prevent recurrence.

5.6 Laboratory Personnel

- Completes appropriate EHS and other lab-specific training, as required by the PI.
- Identifies actual and potential biological hazards and uses proper controls to minimize exposure risk.
- Follows the standard operating procedures, accepted microbiological practices, and other requirements required by the PI and/or the BSM.
- Wears appropriate personal protective equipment, as specified in the Workplace Hazard Assessment form.
- Ensures work areas remain clean, orderly, and in a sanitary condition.
- Secures infectious agents to prevent theft or loss.
- Properly treats, manages and disposes of biomedical wastes.
- Notifies the PI of accidents, injuries, illnesses, spills, or exposures involving biological materials.
- Follows the proper procedures to respond to emergencies or accidents.

5.7 Environmental Health and Safety

- Develops and updates the Biological Safety Manual and other biosafety guidance documents to comply with federal and state regulations and institutional procedures.
- Develops, updates, and provides biological safety trainings.
- Provides consultation on classifications, risk assessments, agent handling, containment, and personal protective equipment.
- Provides oversight of the biosafety training database and verifies training compliance to the appropriate committees.
- Conducts laboratory inspections to ensure compliance with biosafety regulations and standards.
- Properly disposes of biomedical wastes generated from labs and other work areas.

6 BIOSAFETY REQUIREMENTS

6.1 BIOLOGICAL AGENT USE REGISTRATION

Institutions with research and teaching activities are obligated by federal and, or state regulations to know which biological agents are used or stored in their buildings. The identification of the biological agents and biological materials present in an institution is a crucial step in the development of an effective biological safety program.

Principal Investigators are required to register all of their biological agents and materials with Biosafety and the IBC using Husky SMS-SciShield IBC Registration forms. The information provided will be reviewed by the BSO and Biosafety Staff. The IBC will review submissions that involve recombinant or synthetic nucleic acid molecules and agents requiring BSL-2 containment. The review will:

- Determine appropriate risk groups and biological safety containment levels (BSL),
- Identify recombinant or synthetic nucleic acid molecule activities,
- Determine the adequacy of the laboratory for the research activities,
- Determine and establish appropriate biosecurity measures,
- Determine bloodborne pathogens exposure risk,
- Determine and develop necessary and appropriate training, training tools,
- Determine medical surveillance needs,
- Comply with all applicable regulations,
- Determine appropriate shipping and transport procedures,
- Identify related environmental issues (waste handling, facility ventilation), and
- Assist emergency response planning.

Submissions that lack the necessary information for a determination to be made will be returned to the PI with feedback identifying the information required for completion. Registrations must be updated annually and will expire three years from the date of first approval. Biosafety and the IBC must be notified using the amendment process before:

- New agents and materials are brought into the laboratory
- New procedures are conducted with existing agents
- Location of existing work changes
- Agents or materials are shared with other investigators
- Addition of personnel prior to the annual update

Consult the IBC Policy for additional information about the submission and review process.

6.2 Incident Reporting

All accidents, injuries and potential exposures involving biological agents must be reported. An immediate report is necessary to ensure proper care and treatment is given to those involved. A swift and appropriate response could diminish the likelihood of disease transmission. The report will also initiate an investigation. The purpose of this investigation is to review the practices leading up to the incident and will focus on ways to eliminate or diminish any future risks.

Reports are made directly to the supervisor of the work area where the event occurred. Additional reports should be made directly to the individual's supervisor or Department and EHS Biosafety.

Spills and incidents that do not result in an exposure or injury must also be reported if:

- The material is known to contain infectious agents
- The material is known to contain a biological toxin or is a biological toxin
- The material is genetically modified, recombinant or transgenic

Report spills and exposure incidents using the IBC Incident Reporting Form

Accidents, injuries and exposures must also be reported on the *DAS First Report of Injury* WC207 form for anyone employed by the University.

6.3 Connecticut Department of Public Health (DPH) Registration

PIs working with human pathogens are required to register with DPH and Biosafety before they begin work with the pathogens. An initial laboratory survey (inspection) will be conducted by a DPH consultant. A biennial registration renewal and laboratory survey is required thereafter. All registrations and surveys are coordinated by Biosafety.

The Connecticut Department of Public Health (DPH), Public Health Code section 19a-36-A25, states: "Any person, firm or corporation, or the duly authorized agent thereof, operating or maintaining a laboratory in which there is made any examination, determination or test specified in section 19a-36-A26, shall register such laboratory with the state department of health before any such examination, determination or test is made. The carrying on of any of the examinations, determinations or test specified in said section shall be deemed the operating or maintaining of a laboratory."

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

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard)

to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or certain body fluids (bloodborne pathogens.) The Bloodborne Pathogens Standard applies to all employers having employees that are "occupationally exposed" to human blood or other potentially infectious materials.

An employee is considered occupationally exposed if there is "reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of an employee's duties." Other potentially infectious materials include:

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

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

The University's Exposure Control Plan (ECP) describes the methods of determination for occupational exposure. It also includes information about annual training, engineering controls, work practices, personal protective equipment, signs and labels, Hepatitis B vaccination, exposure incident procedures, post-exposure evaluation and follow up and recordkeeping.

6.5 Animal Use

All experimental vertebrate animals listed in the IBC registration must be associated with an approved Institutional Animal Care and Use Committee (IACUC) protocol. The IACUC is charged with responsibility for reviewing the University of Connecticut's program for the humane care and use of animals in research and teaching as described in its Assurance and University Policy. The IBC and Biosafety work with the IACUC when protocols involve the use of any infectious agents, human materials, rsNA and when animals are transgenic. The use of other hazards, chemical or radiological, are reviewed by the relevant EHS section managers. Visit the IACUC website for more information about protocol submission and review.

6.6 Occupational Health and Safety Program for Animal Handlers

The Occupational Health and Safety Program for Animal Handlers (Program) covers all individuals who have occupational and academically related animal contact risks. The Program is available online at the Institutional Animal Care and Use Committee (IACUC) and the Environmental Health and Safety (EHS) web sites. Animal contact includes, but is not limited to, the direct handling or manipulation of animals, alive or dead; transporting animals with or without cages, cage, stall cleaning or washing chores, animal facility custodial activities, and animal feeding or watering chores. Personnel who engage in these activities are covered by this Program, and include Principal Investigators (PI) and their research staff, student employees or students named on protocols and animal care staff. Other University staff that frequent animal housing and come into contact with animals may be included in the program. Isolated one-time animal contact may not require participation in the program. The program requirements are based on the type and frequency of exposure to animals.

The Program includes occupational health services where animal handlers can receive appropriate counseling about the availability of pre-exposure vaccines and receive treatment for exposure to animal allergens, bites, scratches, etc. The Program covers individuals who have animal contact and are listed on animal research protocols conducted by UConn PIs. At each annual protocol renewal, PIs must certify in writing that, to the best of their knowledge, all animal handlers working under that protocol are in compliance with the provisions of the Program.

6.7 Clean Air Devices: Biosafety Cabinets (BSC) and Laminar Flow Benches (LFB)

Clean air devices are equipment that use one or more HEPA filters to deliver clean, nearly particulate free, air to a work surface. Biosafety cabinets (BSC) are designed to protect the worker and the environment from contamination; most protect the product as well. Laminar flow benches (LFB) only protect the product from contamination.

BSCs and LFBs must be certified annually to ensure the unit's ability to perform its intended function. Biosafety funds and coordinates the certifications with a contracted vendor. Each PI is responsible to pay for any services beyond the certification. Units that are not certified annually will be rendered non-functional until such time as a certification is performed.
The purchase or transfer of clean air devices must be approved by Biosafety. PIs must fill out and submit Form J for approval and attach the approved document in Husky Buy or use the EHS Purchase Approval Requisition form in Husky Buy.

The purchase of most LFBs is discouraged because they do not provide any protection to personnel or the environment. There are limited acceptable uses for LFBs.

7 TRAINING REQUIREMENTS

Completion of certain biosafety training is required prior to initiation of your research project or work assignment. The training may include IBC-rsNA specific PI training. Log on to HuskySMS to view required training determined by the lab PI. Register for in person Biosafety training or take any available online training from HuskySMS.

Work Involves	Training Required	Delivery Options
Any biological agent or material	Biosafety General Training Annual Refresher	Online via HuskySMS
Human blood, other potentially infectious materials, including human cell lines	Bloodborne Pathogens Initial Annual Retraining	In person lecture In person lecture or online via HuskySMS
Animal subjects	Biological Safety in Animal Research Biosafety Considerations Working with Rodents or Rabbits in a BSL-1 Setting Biological Safety in Field Research Biological Safety in Farm Animal Research & Production	Online via HuskySMS Online via HuskySMS Online via HuskySMS Online via HuskySMS
Shipping biological agents or materials	Shipping and Transportation of Biological Agents	Online via HuskySMS

8 REGULATORY AGENCIES

This section lists Federal and State agencies that impose biosafety requirements through their guidelines, standards and regulations. Reference will be made to these agencies throughout the Manual.

8.1 NATIONAL INSTITUTES OF HEALTH (NIH)

The Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) address the safe conduct of research and, or teaching activities that involve construction and handling of recombinant or synthetic nucleic acid molecules and organisms containing them.

The Recombinant DNA Advisory Committee (RAC) was established in 1974 to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. The RAC published the initial version of the NIH guidelines in 1976. In 2019, the Novel and Exceptional Technology and Research Advisory Committee (NExTRAC) replaced the RAC as the NIH advisory group for advances in recombinant and synthetic nucleic acid research.

The current NIH guidelines include practices, procedures for research involving recombinant or synthetic nucleic acid molecules. Principal Investigators who are responsible for research and, or teaching activities that involve recombinant or synthetic nucleic acid molecules must include them on IBC registration forms.

8.2 CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) AND THE NIH

The Biosafety in Microbiological and Biomedical Laboratories (BMBL) describes combinations of standard and special microbiological practices, safety equipment and facilities that constitute four levels of biological containment (BSL 1-4) which are recommended for working with a variety of infectious (biological) agents in various laboratory settings. The BMBL also outlines requirements for four levels of animal biological safety containment (ABSL 1-4). The BMBL serves as the basis for UConn's biosafety Manual.
The CDC also issues permits for the importation of infectious agents, materials and host animals into the United States.

8.3 OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (OSHA)

The Laboratory Safety Standard requires employers to write and implement laboratory specific Chemical Hygiene Plans. According to the Laboratory Safety Standard (LSS), a Chemical Hygiene

Plan (CHP) applies to all employees who use hazardous chemicals in the laboratory. Investigators who use biological agents most likely use them with chemicals, some of which are hazardous.

The 1991 Bloodborne Pathogens regulations were created to address healthcare workers concerns regarding occupational health risks caused by exposure to human blood and other potentially infectious materials of human origin. While originally focused on healthcare workers, this standard applies to any personnel who may be exposed to human blood or other potentially infectious materials of human origin, including human cell lines. Refer to the BBP section of the Manual for specific information regarding the BBP standard and how it applies to "occupationally exposed" personnel.

OSHA requires employers to conduct inspections of all workplaces or tasks to determine if hazards are present that would require the use of Personal Protective Equipment (PPE). This process is carried out and documented with a Workplace Hazard Assessment WHA form. PPE is one component to working safely with biohazardous biological agents.

8.4 United States Department of Agriculture (USDA)

USDA issues permits for the possession, use and transport of biological agents and materials important to agriculture and at times, human health. Conditions of these permits vary for each type of agent or material. These conditions often include requirements for containment, handling and disposal of permitted materials.

8.5 Connecticut Department of Energy and Environmental Protection (DEEP)

The state of Connecticut has solid waste management requirements that apply to bio- medical waste (biological, medical, clinical) that may pose or appear to pose a human health risk. DEEP is responsible for the enforcement of the medical waste regulation. The University's Biowaste program was created to comply with this regulation.

8.6 Department of Homeland Security

Federal regulations regarding the use, storage and transfer of certain (select) biological agents and toxins were published starting in 1996. At the federal level, registration and approval of select agents is the responsibility of the USDA-APHIS, CDC National Select Agent Registry (NSAR). Additional information can be found in the Select Agent section of this Manual.

8.7 Other Agencies and Entities

Department of Transportation (DOT), Department of Commerce (DOC), Department of State, Department of Treasury and Department of the Interior all have regulations concerning the movement and shipping of biological agents and materials. The International Civil Aviation Organization and the International Air Transport Association also have shipping requirements. See the Shipping and Transportation of Biological Materials section of this Manual.

Information Systems for Biotechnology's (Virginia Tech) "A Practical Guide to Containment: Plant Biosafety in Research Greenhouses" and The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene's "Arthropod Containment Guidelines" are examples of independent entities whose work has become Biosafety best practices.

9 BIOHAZARDS AND RISK ASSESSMENT

9.1 Biological Agents and Biohazards

Biological agents are preparations made from living organisms and their products. Biological agents include:

- All microorganisms and their toxins
- Viruses and sub-viral particles (including prions)
- Recombinant products (plant, animal, microbial)
- Parasites (microscopic, as well as, macroscopic)
- Cultured human and animal cells and the potentially infectious agents that these cells may contain
- Clinical specimens (human or animal blood, body fluids, cells, tissues, bone)
- Whole animals and tissues from experimentally infected animals
- Allergens (such as molds, microbial spores, and animal allergens)

Biohazards (Biological Hazards) are defined as those biological agents capable of causing disease or death in immune-competent individuals. Biological Safety professionals are required to evaluate all biological agents use because any of the agents may be capable of causing disease or death given favorable conditions.

9.2 RISK ASSESSMENT

The purpose of risk assessment should be risk reduction. Workplace risk (hazard) assessment consists of measuring the risk associated with the use of a particular agent, material, device, or equipment and the consequences of infection, illness, physical injury. To conduct the most appropriate risk assessment, the following should be considered:

9.2.1 Agent hazards:

- Agent origin
- Capacity to infect and cause disease in a susceptible host
- Virulence as measured by the disease severity
- Infective dose
- Routes of entry into a host
- Probable modes of transmission in a laboratory
- Stability in the environment
- Host range
- Availability of prophylaxis and, or effective treatment
- Reports of laboratory acquired infections

9.2.2 Laboratory procedures hazards:

- Parenteral inoculations
- Spills and splashes into skin and mucous membranes
- Ingestion (through mouth pipetting)
- Animal bites and scratches
- Inhalation exposure to infectious aerosols (centrifugation, vortexing, sonication)

The Manual is not meant to provide detailed procedural explanation of laboratory techniques and, or laboratory practices. Instead, the purpose of the Manual is to offer information regarding the variety of controls to consider that address safety concerns. It is up to the Principal Investigator (PI) to determine which controls will be implemented. During periodic biosafety audits, Biosafety staff works with the PI or facility personnel to provide improvements to procedures, as necessary. It is recommended that the PI or their designee perform regular self- audits of their laboratory areas and related facilities. A laboratory Biosafety checklist is available to facilitate self-audits.

9.3 RISK GROUPS

A risk assessment for microorganism use should start with the World Health Organization (WHO) and NIH Risk Group classifications. The classification system assigns organisms to one of four categories of risk based on the agent hazard criteria listed above.

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

Visit the Risk Group webpage for a comparison of international risk group classifications. Information about many biohazardous agents can be found in Section VIII of *Biosafety in Microbiological and Biomedical Laboratories*, Agent Summary Statements and at the Public Health Agency of Canada.

10 BIOSAFETY LEVELS

The CDC-NIH publication, Biosafety in Microbiological and Biomedical Laboratories (BMBL), defines four levels of containment based on the risks assigned to the agents and procedures. Each biosafety level describes the standard microbiological practices, special practices, safety equipment (primary barriers and personal protective equipment), and laboratory facilities recommended to protect personnel and the environment from exposures and contamination. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

10.1 BIOSAFETY LEVEL 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required but may be used as determined by appropriate risk assessment.

Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

10.2 BIOSAFETY LEVEL 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment

Please note: Biosafety level 3 and 4 work requires specially engineered laboratory facilities. These facilities are not available at the University of Connecticut. No work may be conducted with agents requiring BSL 3 or BSL 4 containment at the Storrs or regional campuses.

10.3 BIOSAFETY LEVEL 3

Biosafety Level 3 is suitable for work performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel receive specific training in handling pathogenic and potentially lethal agents, and they are supervised by scientists competent in handling infectious agents and associated procedures.

A BSL-3 laboratory has special engineering and design features

10.4 BIOSAFETY LEVEL 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening diseases that are frequently fatal, agents for which there are no vaccines or treatments, or work with a

related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment are handled at this level until sufficient data are obtained to re-designate the level. Laboratory staff receive specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors are competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor controls access to the laboratory in accordance with institutional policies.

Please see Section IV of the BMBL Laboratory Biosafety Level Criteria for more details.

The BMBL also provides guidance for the use of experimentally infected animals housed in indoor facilities (e.g. vivarium) and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. This information is found in Section V Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities

A table summarizing the laboratory biosafety level recommendations is provided on the next page.

10.5 BIOSAFETY LEVEL 2 ENHANCED/PLUS

There are no standard criteria for this intermediate biosafety level. The enhancements or additions to the standard BSL-2 are dependent on the risk assessment. The most common addition is the requirement that all work with biological agents be done in a biosafety cabinet, which is only recommended for aerosol generating procedures at standard BSL-2. Additions may also include mandatory use of personal protective equipment such as a surgical mask, double gloves and safety glasses. Respiratory protection may be recommended especially in animal facilities.

11 TABLE OF BIOSAFETY LEVEL RECOMMENDATIONS

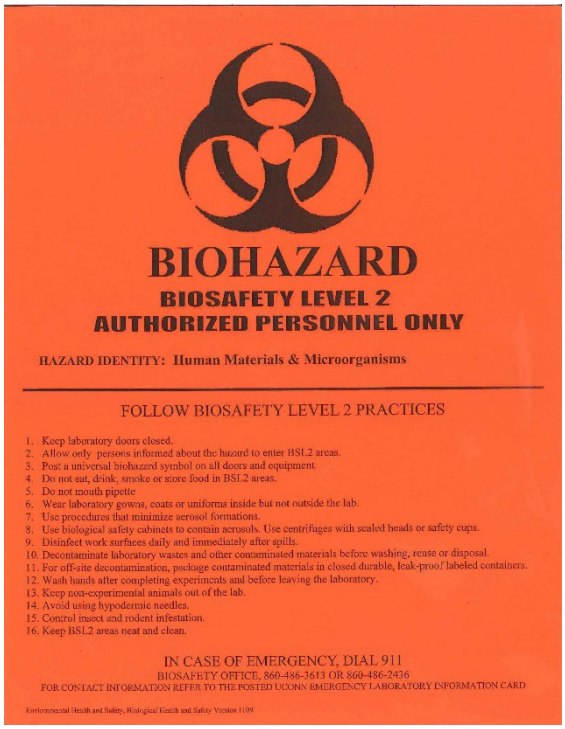
BSL	Agents	Practices	Primary Barriers and Equipment	Facilities
1	Not known to consistently cause diseases in immunocompetent adults	Standard microbiological practices	No primary barriers required. PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory doors, bench and sink required ,windows with screens
2	Agents associated with human disease Routes of transmission include 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: BSCs or other physical containment devices used for all aerosol generating procedures PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: Self-closing doors, sink near exit, autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease or that can be contracted through the inhalation route of exposure	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of laboratory clothing before laundering	Primary barriers: BSCs or other physical containment devices used for all open manipulations of agents PPE: Solid front gowns, scrubs or coveralls; two pairs of gloves, appropriate eyewear, 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 Autoclave preferably in the laboratory
				Hands-free hand washing sink near laboratory exit

4	Dangerous/exotic agents which post high individual risk of aerosol- transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level Related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated prior to removal from the laboratory	Primary barriers: All procedures conducted in BSCs in combination with full-body, air-supplied, positive pressure suit	BSL-3 plus: Entry through airlock, walls, floors, ceilings form sealed internal shell Dedicated non-recirculating ventilation system required Double-door, pass-through autoclave required

12 SIGNS AND LABELS

All persons entering a laboratory must be advised of the actual or potential hazards present and they must be informed of any specific entry, exit requirements. This may be accomplished by the use of signs posted at the entrances to the laboratory. Biosafety will post biosafety level 2 and above labs with a sign incorporating the biohazard symbol, the word biohazard and recommended work practices. The lab must also post the UConn Emergency Information Card.

Biohazard warning labels must be affixed to containers of regulated waste, refrigerators, freezers, incubators and other containers used to store, transport or ship infectious or potentially infectious materials. Biosafety will provide adhesive labels for equipment. The labels shall be fluorescent orange or orange-red with lettering and symbols in a contrasting color (black).



13 LABORATORY PRACTICES

13.1 MICROBIOLOGICAL STANDARD PRACTICES

Whether your experiments are with bacteria, cell lines, body fluids or whole animals, the rules of the lab learned in a Microbiology class will serve to protect you, the materials you work with and the environment from contamination. Standard microbiological practices are the basis for biosafety practices. This list highlights some of these practices. Following sections of the manual will provide more details.

- **Never** mouth pipette. Avoid hand to mouth or hand to eye contact in the laboratory
- **Never** smoke, eat, drink, take medication or apply cosmetics in the laboratory.
- Use aseptic technique.
- Thorough hand washing is essential after handling microorganisms, cells, mammalian tissues or body fluids and prior to exiting the laboratory.
- Wear a laboratory garment, gloves and other recommended items that protect street clothing from contamination in **Biosafety Level-2** laboratories. It is strongly recommended that similar precautions be taken in **Biosafety Level-1** labs as well. Discard laboratory gloves

and remove lab coats upon leaving the laboratory area. Autoclave lab coats if working with cultured human pathogens before sending them to the laundry.

- Use great care and caution when handling syringes and needles, sharps or glassware in the microbiological laboratory. **Never** attempt to recap a used needle prior to disposal. Dispose of syringe needle assemblies in sharp-proof, autoclavable containers.
- Handle human pathogens or infectious materials containing pathogens in certified biosafety cabinets. Use the cabinets wisely, keep free of clutter and materials that block air flow and vent grids.
- **Never** leave pathogenic materials or contaminated labware open to the environment outside the biosafety cabinet. Store all biohazardous materials securely in clearly labeled, sealed containers. Storage units, incubators, freezers or refrigerators should be labeled with the **Universal Biohazard** sign when they house infectious material.
- **Biosafety Level-2** laboratories must be posted with the **Universal Biohazard** symbol and must list infectious agent(s) in use.
- Avoid aerosol-generating procedures when working with pathogenic materials. Pipette mixing, sonication, centrifugation and other seemingly innocuous procedures can produce substantial aerosols. Use safety equipment when aerosol generation is unavoidable.
- **Always** close the laboratory doors when working with pathogenic materials.
- Know the laboratory biosafety plan for handling and the most suitable disinfectant for decontaminating the pathogens you use. Be familiar with the laboratory plan for managing an accidental spill of pathogenic materials. **Always** keep an appropriate spill kit available in the lab.
- Clean up the laboratory work surfaces thoroughly with a proven disinfectant after using pathogenic materials. The containment laboratory **must not** be cluttered in order to permit proper floor and work area cleaning.
- **Never** allow contaminated, infectious waste materials to leave the laboratory non- sterilized as normal trash or to be put in the sanitary sewer without being decontaminated. Use incineration or autoclaving at adequate temperature (121° C), pressure (18 psi) and time (based on the size of the waste load) and always use a sterile indicator tag to denote sterilization. Expose all materials being sterilized to the flowing steam principle.
- When shipping or moving pathogenic materials to another laboratory, **always** use DOT approved, leak-proof, sealed and properly packed containers (primary and secondary containers).
- Report all accidents, occurrences and unexplained illnesses to your work supervisor.
- Understand the pathogenesis of the biohazards with which you work.
- Protect your fellow workers and the public from the pathogens you use.

- Think **safety** at all times during laboratory operations. The greater the hazard, the greater care that must be exercised. If you do not understand the proper safety procedures or the use of the appropriate safety equipment, do not work with the biohazard until you receive proper instruction. Seek the advice of the appropriate individuals.

13.2 HAZARD CONTROLS

Elimination and substitution are two of the most effective hazard controls available. The first may not be practical with biological agents. Often to eliminate the hazard, the agent must be dead. Substitution may be possible if a less hazardous variety, species, strain exists or has been engineered. If the hazards cannot be eliminated or diminished, then a way to contain the hazard and shield the worker from exposure must be explored in the form of engineering controls. Administrative Controls should be in place before addressing Engineering, Work Practice and Personal Protection Controls. These controls include the elimination and/or substitution of hazardous agents and procedures, creation of standard operating procedures (SOP), laboratory and agent specific training and appropriate supervision of workers during hazardous or potentially hazardous procedures.

13.3 ENGINEERING CONTROLS

Engineering Controls include equipment, devices or supplies that reduce the risk of occupational exposure by removing the hazard or isolating the worker from the exposure. Biological safety cabinets, autoclaves, safety centrifuges, medical sharps containers, splash guards, mechanical pipetting devices, and self-sheathing needles, needle devices are examples of engineering controls. Supervisors are responsible for providing the necessary engineering controls in the workplace.

Utilize appropriate Engineering Controls whenever possible. Engineering controls are most effective when they are combined with good work practices. Equipment that is an engineering control will require preventive maintenance or periodic replacement to provide worker protection. It is the direct responsibility of the supervisor to ensure these controls operate properly.

Equipment such as biological safety cabinets, safety centrifuges and mechanical pipetting devices are to be decontaminated immediately (or as soon as feasible) when overtly contaminated, or after a spill of biological agents, blood or other potentially infectious materials. Engineering Controls should also be routinely decontaminated at the end of the work shift.

13.4 Engineering Controls to Reduce or Eliminate Needle stick Injuries

Needle-sticks may occur in situations where needles require manipulation or disassembly. Devices or systems such as Catheter Safety Systems, I.V. Access Safety Systems, Needle Protection Devices, Air Bubble Removal Devices, Syringe and Needle Shields reduce the need to use needles and lessens the danger of accidental needle-sticks. There are also safety sharps devices such as retractable lancets and disposable scalpels with safety features. Contact EHS-Biosafety section with any questions about safer sharps devices.

Sharps contaminated with biological agents, human blood, or other potentially infectious material and capable of puncturing the skin must be disposed of in sharps containers distributed by EHS-Waste Management section. Waste items that can puncture or tear plastic bags are considered sharps and should also be disposed of in sharps containers.
Sharps disposal containers are to be used to contain and discard used and unused sharps waste. Containers for sharps disposal should be easily accessible and located as close as possible to the immediate area where sharps are used or found. Sharps containers should be puncture-resistant, closeable and leak-proof on the sides and bottom. Contact EHS Waste Management section for sharps containers.

Proper use of sharps containers eliminates the need to recap, bend, break or manipulate sharps waste by hand. Procedures that absolutely require activities such as recapping of needles must have an approved “Recapping Approval” letter signed by the BSO. Contact the BSO, EHS-Biosafety section for information about the recapping approval procedure.

Specific examples of sharps include: needles, scalpel blades, hypodermic needles, syringes (with or without attached needles), Pasteur pipettes, disposable pipettes, razor blades, blood vials, test tubes, needles with attached tubing, pipette tips, broken plastic culture dishes, unbroken glass culture dishes regardless of the presence of infectious agents, and other types of broken and unbroken glass waste materials including microscope slides and coverslips. (Waste items that can puncture or tear plastic bags are considered sharps). **PLEASE NOTE**: Sharps containers that are in use are to be kept in an upright position and closed before removal from the laboratory to prevent spillage or protrusion of contents during handling, storage or transport. Sharps containers should not be allowed to overfill before replacement. All medical sharps containers that are provided by EHS have a “Fill To” line on the outside.

Reusable sharps should be placed in puncture-resistant containers that are leak-proof on the sides and bottom. Reusable sharps containers should be red or labeled with the biohazard symbol and the word "Biohazard" in the legend

Reusable sharps are to be placed in reprocessing containers immediately or as soon as feasible after use. Contaminated reusable sharps shall be stored or processed in a way that does not require a worker to reach into the container by hand, risking the possibility of an injury or needle-stick.

13.5 ENGINEERING CONTROLS TO CONTAIN SPILLS

Use of **plastic backed towels, bench coats or bench diapers** in conjunction with the biohazard symbol provides a flexible, clear definition of work areas when potentially infectious materials are in use. They absorb potentially infectious material in the event of a spill and facilitate clean-up when work is completed. They can be taped to laboratory benches or placed inside biological safety cabinets. They should be placed plastic side down so the absorbent side can soak up any inadvertent spills. Dampening the absorbent side with tuberculocidal disinfectant is an added infection control measure. **PLEASE NOTE**: Decontaminate plastic backed towels, bench coats or diapers before discarding. Replace them when visibly soiled or at the end of the work shift. Do not place notebooks, pens and other common use items in the defined biohazard work area.

Spill trays used in conjunction with the biohazard symbol clearly define work areas where potentially infectious materials are in use. They are available in a variety of sizes and shapes. The chemical resistant surface facilitates chemical decontamination. Because spill trays are reusable, the volume of medical waste generated each day may be reduced. Long term use of spill trays may be more cost effective than plastic backed towels or diapers. Limitations of spill trays include: tray rigidity which may be cumbersome for certain procedures; infectious materials spilled on the tray may splatter or form aerosols.

Specimen containers and specimen transport bags are to be used to prevent leakage of human blood or other potentially infectious materials during collection, handling, processing, storage, transport or shipping. Specimen containers include test tubes, freezer vials, etc. Specimen containers and transport bags should be labeled with a biohazard symbol and the word "biohazard" in the legend when the specimens leave the facility or when the specimen containers are not readily identifiable as containing specimens. Some specimen bags have outside pockets to help keep accompanying paperwork from being contaminated. Handle all specimen transport bags with appropriate disposable gloves. Assume the specimen container or the paperwork in outer pocket may be contaminated. **PLEASE NOTE**: Examine all containers and bags for cracks, chips, tear holes or other flaws **before use**. Flawed containers or bags may break or leak. Do not use defective containers or bags - discard them through the medical waste stream. Primary containers that become contaminated on the outside shall be placed

within a secondary container. Secondary containers will then be subject to all biohazard labeling requirements. Primary containers that can be punctured by the specimen are to be placed in a puncture-resistant secondary container. The secondary container will then be subject to all labeling requirements.

13.6 Engineering Controls to Help Contain Splashes and Aerosols

Vacuum line chemical traps and filters prevent suction of human blood and other potentially infectious materials into the vacuum lines. The trap systems also prevent vacuum lines from clogging with non-infectious material. Laboratory workers protect maintenance personnel and vacuum line by installing and maintaining vacuum line traps and filter systems. **PLEASE NOTE**: Filters are to be placed between the chemical traps and the vacuum line. Do not allow trap vessels (flasks or bottles) to overfill. Filters are to be examined and replaced if clogged or if liquid makes contact with the filter. Confirm all connections or seals are tight to ensure the vacuum is adequate. Spent filters are to be discarded in the medical waste stream. There are now a variety of inexpensive HEPA in-line filter traps on the market. Contact the EHS –Biosafety section for information regarding vacuum line filters.

Splash guards are clear plastic shields that prevent potentially infectious materials from splashing onto laboratory workers. They protect against splashes, but not aerosols and they will block a splash from one direction only. Workers should avoid leaning over splash guards.

Biological Safety Cabinets (BSCs) are Clean Air Devices (CAD) that offer personal, product and environmental protection. They prevent biohazards from contaminating the worker by confining contaminants within the operating cabinet and removing them through High Efficiency Particulate Air (HEPA) or Ultra High Efficiency Particulate Air (ULPA) filters. The intake air must pass through the filter before flowing into the BSC work area. The exhaust air is also filtered by a HEPA or ULPA filter. Any aerosols generated within the cabinet work area are contained within the BSC. Proper air flow is essential to the containment nature of BSCs and other CAD. BSCs **must** be certified by a qualified and reputable certifier after installation, and before use, after being relocated and at least annually thereafter. BSCs in use or with uncertain or undetermined usage are to be professionally decontaminated before relocation, storage or service to the interior of the unit. Contact EHS Biosafety section to coordinate BSC and other CAD certifications. Consult the manufacturer's instruction manual for information concerning the BSC, CAD work zone, protective air curtain and other performance-affecting concerns.

Additional considerations for BSC use include the following:

Small table-top centrifuges, sonicators, stirrers and shakers may be operated within a biological safety cabinet to protect workers from any aerosols generated during their operation and when opening pressurized containers.

Biological safety cabinets with microscope modifications are available when the use of a microscope in a clean environment is necessary.

Many BSCs are equipped with one or two germicidal Ultra-Violet (UV) lamps. The germicidal effect of the U.V. lamp is affected by time of exposure, distance, presence of dust or debris, and the UV lamp intensity. The lamp needs periodic cleaning and although it maintains a visible blue glow throughout its lifetime, a UV lamp may have no germicidal effect. Routine BSC work surface decontamination is as effective as the use of germicidal lamps.

PLEASE NOTE: Ultraviolet lamps may damage eyes, skin and laboratory equipment. These hazards have led the NIH, CDC, NSF, ANSI and the American Biological Safety Association International to **discourage their use in biosafety cabinets**. If they are present, UV lamps are to be turned off while the room is occupied and the BSC or other CAD is in use.

Mechanical pipetting devices are to be used in place of mouth pipetting or mouth aspiration. The proper use of mechanical pipetting devices eliminates or reduces the risk of contamination of the mouth, hands and work environment. **PLEASE NOTE**: Although mechanical pipetting devices used with biohazardous agents are decontaminated after use, maintain them labeled with biohazard labels.

Centrifuge safety cups or buckets are designed to retain the contents of the centrifuge tube in the event of breakage or leakage. Open these inside a BSC to contain the release of any potential aerosols. The contents may be under pressure.

Safety Centrifuges (Centrifuges with automatic locking mechanisms or solid lids) prevent the centrifuge lid from being opened while the rotor is still in motion or prevent aerosol release while the centrifuge is in operation. The locking device releases after the centrifuge head has stopped revolving.

Safety Blenders and Safety Sonicators are designed to contain aerosols during operation. Open them inside a BSC or behind a splash guard to contain aerosols or block the release of contents. The contents may be under pressure.

13.7 Engineering Controls for Contamination Control

Chemical disinfectants destroy disease causing agents on inanimate surfaces. Chemicals proven to kill Mycobacterium tuberculosis on surfaces, called tuberculocidal disinfectants are considered to be broad spectrum disinfectants. A 10% solution of standard (5.25% NaOCl) household bleach or 2% Lysol (commercial) are examples of tuberculocidal disinfectants. **EPA registered disinfectants are required in labs using human materials.**

Some materials of human origin and any materials that may contain prions such as Creutzfeldt-Jakob (CJ) infectious agent, require multiple decontamination steps to achieve inactivation. Prions are resistant to common disinfectants.

Ethanol or isopropyl alcohol (70%) have limited effects as disinfectants and are not generally considered to be disinfectants. These alcohols are effective cleaners or sanitizers and may be used after an appropriate disinfectant.

The following criteria will help in the selection of a disinfectant or sterilant:

- The product must be effective against all organisms present in the laboratory
- The product must be compatible with surfaces/materials being decontaminated
- The product must be able to maintain sufficient contact time to inactivate the organisms
- The product must have sufficient stability in its diluted form to achieve inactivation

Contact EHS-Biosafety section with questions about the selection of disinfectants.
Physical contamination controls use low temperature, high temperature (either dry or moist heat), incineration, osmotic pressure, sonic and ultrasonic waves, ultraviolet light, x or gamma rays to achieve their effect. Decontamination renders an item safe for further handling.

Temperature above a maximum will exert a killing effect whereas temperature below a minimum exerts a static effect (microorganisms lose the ability to grow).

Moist heat has greater penetrating power than dry heat. One of the most effective physical contamination controls is steam sterilization (autoclave) which generates moist, high temperature, pressurized steam within a sealed chamber.

Autoclaves can sterilize all items that are heat stable (not damaged by steam or high temperature). In gravity autoclaves, decontamination is achieved at 250□F (121□C), 15 to 18 lb. pressure. In vacuum autoclaves, decontamination is achieved at 270□F (132□C), 27 to 30 lb. pressure at a shorter exposure time.

Personal protective equipment (PPE) such as rubberized aprons, full face shields and heat and liquid resistant gloves should be worn when operating autoclaves. The wearing of such PPE is an OSHA requirement.

Reusable glass and other labware can be decontaminated in hot air ovens. Higher temperatures and longer time periods are required to achieve decontamination. Hot air decontamination is achieved at 160^0- 180^0 C in 1-4 hours. A minimum of 2 hours is required to destroy spores. The effectiveness of hot air ovens is increased if the oven has a circulating fan.

Whatever the temperature and time, each appropriately packaged item in a load must be placed so that steam penetrates into, or heated air flows freely among all materials to be decontaminated. Tightly sealed or stoppered materials may not be effectively decontaminated and may become dangerously pressurized causing injury when removed.

A routine autoclave maintenance program should be in place. Routine physical controls such as pressure gauges and thermometers are to be considered secondary methods of ensuring a sterilization cycle. Regular chemical monitoring of temperature and periodic biological monitoring must be performed and recorded. Biological indicators (biological monitoring) at locations throughout the autoclave are the best indication of sterilization. The use of autoclave tape is not a form of biological monitoring. Contact EHS-Biosafety section for information regarding effective biological monitoring.

PLEASE NOTE: State of Connecticut DEEP (Department of Energy and Environmental Protection) biomedical waste regulations require that autoclaves used to treat waste be evaluated with respect to effectiveness of the sterilization process, temperature and pressure at least once during every forty hours of operation. A log recording temperature and pressure readings and results of biological tests is also required. The University's biomedical waste terminal treatment is performed by a contractor.

When autoclaves or hot air ovens cannot be used, an alternative method such as chemical decontamination can be employed (see the Work Practices section).

13.8 Labeling Contaminated Equipment for Repair or Discard

Potentially contaminated and contaminated equipment sent out for repair or discard must be decontaminated as thoroughly as possible. Attach a tag indicating when the equipment was decontaminated, what product was used to decontaminate, and the name of the person who performed the decontamination. If the equipment cannot be completely decontaminated, decontaminate all areas possible, attach a biohazard label to the equipment and indicate, in

some fashion, those areas of the equipment which remain contaminated. The label and indicator should be easily readable to all affected personnel.

14 WORK PRACTICE CONTROLS

Work Practice Controls reduce the likelihood of worker exposure to infectious agents by altering the manner in which a task is performed. The protection provided by Work Practice Controls is based upon worker behavior and attitude.

Work Practice Controls ensure that Engineering Controls and Personal Protective Equipment are used effectively. Work Practice Controls protect others from exposure to pathogens in the work area or facility. Work Practice Controls reduce possible cross contamination and improve the quality of the work performed. Routine safe work practices provide a margin of safety for unrecognized hazards.

Remember: Safety is a shared responsibility. Your attitude and work practices are critical for your own health and safety, and for the welfare of those around you.

Work Practice Controls include the following:

14.1 UNIVERSAL (STANDARD) PRECAUTIONS

An overall approach to reduce the occurrence of an exposure incident in the workplace that incorporates Engineering Controls, Work Practices and Personal Protective Equipment

14.2 USE OF ENGINEERING CONTROLS

Make sure you understand how to properly use and maintain the engineering controls in your facility. Examine and maintain engineering controls before and after use, and before decontamination. If you supervise other workers, you are responsible to ensure that they understand which engineering controls to use and how to use them.

14.3 GENERAL WORK PRACTICES

Organize and plan work procedures and keep an uncluttered work space.
Always make sure all necessary materials are at hand. Keep the appropriate disinfectant, such as 10% household chlorine bleach and paper towels nearby in case of a spill.
Remove and leave laboratory coats, gowns or smocks in the clinical or laboratory areas before going to general access areas such as lunchrooms/cafeterias, libraries and administrative offices.

Beards or mustaches may pose a risk in workplaces with potential airborne contamination. Facial hair retains particulate contamination more persistently than clean shaven skin. Beards and mustaches must be closely trimmed and carefully shaped if face masks or respirators are worn. Clean shaven faces improve the fit of face masks and respirators when required.

Eating, drinking, smoking, vaping, applying cosmetics, lip balm and handling contact lenses are prohibited in potentially contaminated work sites. Hand creams and lotions are permitted when not applied to the face. **Use non-petroleum based hand creams only**.
Petroleum based hand creams compromise glove integrity at the microscopic level.

14.4 Hand Washing

Washing minimizes the hazard of infectious agents by physically removing microbes from body surfaces. Hand washing removes microorganisms from hands/skin that may become contaminated during manipulation of specimens, equipment, supplies, and contact with patients and environmental surfaces.

Each research or teaching laboratory area should have readily accessible hand-washing facilities.

Wash hands with soap and running water for at least 20 seconds immediately (or as soon as feasible) after removing gloves and other personal protective equipment. Wash hands when leaving the work area for general access areas such as lunchrooms, libraries, and administrative offices.

Wash any other skin areas with soap and water, and flush mucous membranes with water immediately (or as soon as feasible) following contact with blood or other potentially infectious materials.

Those working in field situations should use hand sanitizer in conjunction with a clean cloth or paper towels, or antiseptic towelettes. **PLEASE NOTE**: Hands are to be washed with soap and running water as soon as feasible even when hand sanitizers have been used.

14.5 Handling Disposable Needles and Syringes

A hypodermic needle and syringe should only be used for parenteral injection and aspiration of fluids from humans, laboratory animals and diaphragm bottles. Use extreme caution when handling needles and syringes. Use needle-locking syringes or disposable syringe-needle units

(i.e. the needle is integral to the syringe) as much as possible. Avoid autoinoculation and aerosol generation during use and disposal

Contaminated needles are not to be sheared, bent, recapped or removed unless the supervisor can demonstrate to the BSO that no alternative is feasible or that such action is required by a specific research procedure. Exemption from recapping prohibition must be done in writing to the BSO. If recapping exemption is approved, then such recapping or removal of a needle must be accomplished through the use of a mechanical device or a one handed technique. Finger or hand shields offer full protection of the hand holding the needle sheath from accidental puncture. The one hand scoop method is a technique where the hand holding the sharp is used to scoop up the cap from a flat surface.

All needles and syringes are to be discarded promptly into appropriate sharps containers after use.

14.6 Reusable Sharps

Contaminated reusable sharps should be placed in labeled puncture resistant containers as soon as possible after use and stored or processed in a way that does not require workers to reach into the reprocessing container by hand and risk a needlestick or other injury.

14.7 Footwear

Always wear close-toed shoes. Close-toe shoes completely cover your feet, not just your toes. Sandals or open-toed shoes do not provide adequate foot protection and are inappropriate in laboratory or animal care areas. A dedicated pair of work shoes may reduce the amount and type of contamination introduced into the workplace by street shoes. This practice can also minimize the possibility of bringing microbial contamination from the workplace into the home. Steel-toed shoes are recommended when working in farm animal care areas.

14.8 Splash and Aerosol Control

All procedures involving infectious or potentially infectious materials are to be performed in a manner that minimizes splashing, spraying, spattering and generation of droplets. This precaution decreases the chances of direct personal exposure and reduces the contamination of bench tops, instruments or other surfaces in the work area.

Liquid cultures of infectious material, sealed ampoules and Vacutainers are best opened in a biological safety cabinet (BSC). If a BSC is not available, use a splash guard or other engineering control.

14.9 HOUSEKEEPING FOR LABORATORY WORKERS

Laboratory workers are responsible for certain housekeeping activities.

Work surfaces should be decontaminated immediately (or as soon as feasible) when overtly contaminated, or after a spill of infectious or potentially infectious materials. Work surfaces should also be cleaned at the end of the work shift.

General trash receptacles should be inspected daily to ensure that regulated sharps are not inadvertently discarded in the general waste stream. Improperly discarded sharps can result in puncture wounds and cuts to housekeepers and other support staff.

Contaminated broken glassware should not to be picked up directly by hand. Use engineering controls such as a brush and dust pan, tongs or forceps to facilitate clean up.
After use and before storage or discard, decontaminate engineering controls with a tuberculocidal disinfectant. **Vacuum cleaners are not appropriate for clean-up of broken, contaminated glass.**

Equipment that may become contaminated with infectious or potentially infectious materials shall be decontaminated: when visibly contaminated; at the end of the work shift; and prior to servicing or shipping.

Complete decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. Decontaminate the equipment as well as possible (flushing lines or wiping down the exterior) and affix a label to the equipment before sending for repair. The label shall indicate what portions of such equipment remains contaminated and include the biohazard symbol, and the term "biohazard". The label shall convey this information to all affected workers (service representatives, manufacturer, etc.). Heavily soiled equipment that is also contaminated should be prewashed before being decontaminated. Most disinfectants or sterilant cannot sufficiently penetrate organic material present on heavily soiled equipment.

Water baths and water bath sonicators used for inactivating, incubating or testing infectious substances should contain a disinfectant or other microstatic agent to minimize bacterial, fungal or algal growth. Change the water periodically or whenever growth is observed.

Freezers and refrigerators should be checked periodically. Promptly remove any broken vials, ampoules or tubes containing infectious material and decontaminate the inside of the freezer or refrigerator.

14.10 Chemical Sterilization and Disinfection

Always clean surfaces and equipment before using a chemical disinfectant or sterilant. **PLEASE NOTE:** If you use chemical agents to disinfect, decontaminate, sanitize surfaces, equipment, microbial, cell culture or skin surfaces, you should be familiar with the difference between disinfectants, antiseptics and sanitizers in order to use the products correctly and effectively. OSHA requires EPA registered disinfectants or 10% household chlorine bleach to disinfect surfaces contaminated with human blood or other potentially infectious human materials. Household chlorine bleach must have a concentration of 5.25% w/v (100,000 ppm) sodium hypochlorite. A 10% solution results in greater than a 0.5% w/v sodium hypochlorite, which inactivates bloodborne pathogens.

Read the label on your disinfectant, antiseptic or sanitizer container to determine the appropriate dilution necessary to decontaminate environmental and body surfaces against bloodborne and other pathogens.
Listing of EPA registered products

15 BIOLOGICAL WASTE

At The University of Connecticut, biological waste is defined as infectious waste, pathological waste, chemotherapy waste and the receptacles and supplies generated during its handling and, or storage. This definition is in accordance with the definition of biomedical waste as defined by the Connecticut Department of Energy and Environmental Protection (DEEP). It is further defined as waste that, because of its quantity, character or composition, has been determined to require special handling.

15.1 Categories of Infectious Waste

1. **Cultures and stocks**: Agents infectious to humans and associated biologicals, waste from biological production, live and attenuated vaccines and anything used to contain, mix or transfer agents. This includes but is not limited to petri dishes, pipettes, pipette tips, microtiter plates, disposable loops, eppendorfs and toothpicks.
2. **Human blood, blood products and infectious body fluids**: This category includes blood that is not contained by a disposable item or is visibly dripping, serum, plasma, and other blood products or non-glass containers filled with such discarded fluids. It further includes any substance which contains visible blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid and pericardial fluid. Glass containers filled with such discarded fluids shall be considered sharps. Intravenous bags which did not contain blood or

blood products shall not be considered a blood product. Dialysates are not considered blood or body fluids.

3. **Sharps**: needles, scalpel blades, hypodermic needles, syringes (with or without attached needles) and needles with attached tubing **regardless of contact with infectious agents are considered by EPA and DEEP to be REGULATED MEDICAL WASTE**. **Other sharps**: pasteur pipettes, disposable pipettes, razor blades, blood vials, test tubes, pipette tips, broken plastic culture dishes, glass culture dishes and other types of broken and unbroken glass waste (including microscope slides and cover slips) that may have been in contact with infectious material. Items that can puncture or tear autoclave bags.
4. **Research animal waste**: contaminated carcasses, body parts and bedding of animals that were intentionally exposed to infectious agents during research or testing are disposed of as biowaste. Animal carcasses and body parts not intentionally exposed to infectious agents during research or testing are disposed of through a separate waste stream and vendor.
5. **Isolation waste**: biological waste and discarded material contaminated with body fluids from humans or animals which are isolated because they are known to be infected with a highly communicable disease (biosafety level 4 agent).
6. Any untreated material collected during or resulting from the cleanup of a spill of infectious or chemotherapy waste.
7. Any waste mixed with infectious waste that cannot be considered as chemical hazardous waste or radioactive waste.

15.2 Potentially Infectious Material

Potentially infectious material is defined by the OSHA Bloodborne Pathogens Standard as:

1. Human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids
2. Any unfixed tissue or organ (other than intact skin) from a human (living or dead) including cell or tissue cultures and
3. HIV-containing cell or tissue cultures, organ cultures and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

15.3 "LOOK- A - LIKE" INFECTIOUS WASTE

Laboratory materials that can be used to contain, transfer or mix infectious agents but have been used with non-infectious agents. For example: disposable micropipette tips may have transferred sterile water or broth, but an identical tip in the same laboratory may have transferred an infectious agent. In the trash you could not distinguish between them. These "look- a -like" materials must be handled as infectious waste if the facility routinely generates infectious or potentially infectious biological waste or is engaged in a temporary project that generates infectious or potentially infectious biological waste.

Biowaste treatment and disposal procedures can be found on the Regulated Waste Management page.

16 PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment is to be used to provide additional protection when an occupational exposure remains after Engineering and Work Practice Controls are in place.

Barrier Precautions is another term for the use of PPE. PPE is used to prevent biological agents, blood or other potentially infectious materials from making direct contact with an employee's clothing or body. The type and amount of PPE required depends upon the task to be performed and the type of anticipated exposure.

Supervisors are responsible to discuss with their employees the type and proper use of PPE needed to perform job tasks safely. In addition to being an OHSA requirement, the Workplace Hazard Assessment (WHA) is a useful tool to use to determine PPE necessary for job tasks.

16.1 GENERAL GUIDELINES FOR PERSONAL PROTECTIVE EQUIPMENT

- Individuals who supervise occupationally exposed workers are required to provide all appropriate PPE at no charge to the employee and are responsible for cleaning, repairing, disposing and replacing all PPE. Supervisors should be aware of their responsibilities regarding PPE maintenance and replacement.
- PPE should be easily accessible and of proper size to meet individual fit needs.
- PPE should not permit biological agents, blood or other potentially infectious materials to pass through or to reach the employee's outer or inner clothing (including uniforms), skin, eyes, mouth, or other mucous membranes while PPE is used under normal conditions.
- Hypoallergenic gloves, glove liners, powder-free gloves or different glove brands will be provided to employees who exhibit allergic reactions to the gloves normally provided.

- All PPE is to be removed prior to leaving the work area for common areas such as cafeterias, break rooms, offices, etc. PPE should be placed in an appropriately designated area (waste accumulation area) or container, for storage, washing, decontamination or disposal.
- Garments penetrated by biological agents, blood or other potentially infectious materials are to be removed immediately or as soon as feasible. Such garments are to be laundered using a commercial laundry service or discarded.
- Supervisors are responsible for taking necessary measures to ensure their employees adhere to safety and health procedures.
- Personal Protective Equipment includes disposable (Single Use) gloves, rubber utility gloves, protective body clothing (gowns, coats, jumpsuits, aprons), face and eye protection, nose and mouth protection, respiratory protection, surgical caps, hoods or head covers, and shoe protection.

16.2 HAND PROTECTION

- Disposable single-use gloves should fit properly and be long enough to prevent exposure at the wrist or lower arm, and be comfortable. They are to be inspected prior to use.
- Factors to consider when using gloves:
- Glove quality can vary with age, manufacturer and construction materials.
- Gloves should be replaced as soon as feasible if contaminated, torn, punctured or the integrity of the glove barrier is compromised.
- Disposable gloves do not protect against injuries from needles or other sharp objects.
- There are cut-resistant and needle-stick resistant reusable gloves such as KEVLAR AND DYNEEMA.
- Contaminated gloves should be discarded in the medical waste stream.
- Reusable rubber utility gloves may be more desirable than disposable gloves when performing certain procedures such as cleaning and may be washed and reused as long as the integrity of the glove is not compromised.

Avoid grasping the outside of a contaminated glove with bare hands.

Disposable and reusable gloves should be removed when handling doorknobs, telephones, portable electronic devices and elevator buttons.

Always wash hands thoroughly with soap and water after glove removal.

16.3 Body Protection

Gowns, labcoats, jumpsuits and other protective body clothing protect the wearer's clothing and skin from contamination. As with all PPE, the type of clothing needed will depend on the task being performed and the degree of exposure anticipated.

Solid front, wrap-around clothing offers better protection than pull-over type clothing or clothing with front closures. Spills and splashes occur most often in the chest or lap area. The contaminated surface must be touched during removal of a front closing jacket. The contaminated portion often ends up in the wearer's face during removal of pull-over clothing. Many workers do not button up the front closing jackets. This behavior leaves street clothing exposed. If front closing jackets are worn, strict measures shall be implemented to ensure the clothing is closed at all times when performing procedures or tasks that may cause exposure.

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

Plastic, vinyl or rubber aprons are usually worn over other protective body clothing when extra protection is desired. Aprons are generally used for protection against liquid spills, splashes or spurts of biological agents, blood or other potentially infectious materials. Plastic, vinyl or rubber aprons may also be used to provide protection from steam and hot water in locations such as animal handling facilities, autoclave rooms and laboratory glass washing rooms.

Shoe covers or boots should be worn when gross contamination is reasonably anticipated. Circumstances where shoe protection may be necessary include animal rooms, surgery and necropsy rooms, and clinical procedure areas. Shoe covers are required to prevent contamination migration and direct and indirect transmission.
Protective clothing is to be removed as soon as feasible if contaminated or penetrated by biological agents, blood or other potentially infectious materials, and autoclaved or otherwise disinfected before being discarded or laundered

16.4 Face and Mucous Membrane Protection

ANSI Z87.1 certified protective eyewear and masks should be worn whenever there is potential for the generation of splashes, spray, splatter or droplets of biological agents, blood or other potentially infectious material in the eyes, nose, mouth or other facial areas.

Eye protection may prevent damage to the eye in addition to preventing exposure to pathogens. Certain disinfectants and other chemicals can damage the eye or cause blindness if splashed in the eye. Eye protection, such as goggles, provides protection on the sides and tops of the eyes.

Prescription glasses provide no protection in the laboratory and should not be substituted for required eye protection. The use of contact lenses may increase the risk of eye damage because infectious agents and other microbes may become trapped between the contact lens and the cornea. The liquid between the contact lens and cornea is an excellent growth medium for microorganisms. If contact lenses must be worn, barrier eye protection is to be worn.

Surgical masks are generally protective against droplets, splashes and sprays. Some surgical masks are available with attached eye shields. Masks shall cover both the nose and the mouth and shall be positioned and secured on the face to minimize gaps or spaces between the face and mask.

Head covers are worn when gross contamination or splashing on the head is reasonably anticipated. These situations may arise when performing necropsies, surgery or working in animal facilities. Head covers are required to prevent contamination migration and direct and indirect transmission.

Respiratory Protection: Filtering face piece (N95 respirators) are a type of air purifying respirator and like most respirators require compliance with the OSHA Respiratory Protection regulations. OSHA Respiratory Protection compliance includes medical evaluation, respirator training and fit testing. For more information refer to the University's Respirator Program.

Decontamination of Personal Protective Equipment
Disposable gloves shall not be washed or decontaminated for re-use. Disinfecting agents (including soap and water) often cause deterioration of glove material. Washing with surfactants can result in "wicking" or enhanced penetration of liquids through the gloves. Wicking can transport potentially infectious materials to the skin inside the glove.
Rubber utility gloves may be decontaminated and discarded in the general waste stream. Contaminated utility gloves shall be discarded in the medical waste stream.
Employees are not to take laboratory clothing home for laundering. Laboratory clothing is to be laundered commercially or at the work site. Contaminated laboratory clothing should be autoclaved or otherwise decontaminated before being discarded or laundered.

Reusable face protection (face shields) and mucous membrane protection (goggles, safety glasses) are to be decontaminated with the disinfectant available or 10% household chlorine bleach.

17 SELECT AGENTS AND TOXINS

Biological select agents and toxins are regulated by the Department of Homeland Security through regulations promulgated under Health and Human Services and the Department of Agriculture-Animal and Plant Health Inspection Service. The Federal Select Agent Program oversees the possession, use and transfer of biological select agents and toxins, which have the potential to pose a severe threat to public, animal or plant health or to animal or plant products. A full description of the program and its requirements may be found at the Select Agents website.

Institutions and entities must register with and be inspected by the Select Agent Program if they possess, use, or store any of the listed agents. Registration is not required for diagnostic laboratories that destroy or transfer specimens which test positive for a Biological Select Agent or Toxin as long as they comply with reporting requirements. Registration is not required if individual Principal Investigators possess and use exempt quantities of Select Agent Toxins.

Select Agent List
Exempt Toxin Amounts

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

HHS Toxins [§73.3(d)(3)]	Amount
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	200 mg
Diacetoxyscirpenol (DAS)	10,000 mg

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

Exempt quantities of Select Agent toxins, attenuated or excluded Biological Select Agents and any genetic sequences or plasmids from Biological Select Agent must be registered with Biosafety. Purchases of exempt toxins must be approved by the Biosafety Officer. Please refer to Working with Exempt Biological Select Agents and Toxins Policy.

18 SHIPPING, RECEIVING AND TRANSPORTATION OF BIOLOGICAL MATERIALS

Research samples and materials may need to be brought to another location on campus or to another campus entirely. Samples may need to be brought to campus from offsite collection locations. In these circumstances, materials are transported by University personnel in a vehicle. Transporters must adhere to the principles of triple packaging. A leak-proof primary container is placed inside a leak-proof secondary container which is then placed inside a rigid outer container. The containers should be appropriate for the types of samples and take into consideration any temperature requirements. Please refer to the Transportation of Biological Samples policy.

Biological agents and materials may need to be shipped to a laboratory in another state or country. When the services of a shipping company are required to send them, the person shipping the materials must be trained and certified. The training provides information about how to comply with United States laws and international regulations. Shipments must be classified, packaged, marked, labeled and documented by trained individuals. Permits and licenses from Federal agencies may be required to move materials between states or out of the country. Please refer to the Export Control site. The University or the receiving institution may require a material transfer agreement. Please refer to the Material Transfer Agreement site.

Permits and material transfer agreements may be required for you to receive biological agents and materials. It is the responsibility of the individual receiving the materials to apply for any necessary permits from agencies such as USDA-APHIS and the CDC. It is also their responsibility to register these materials with Biosafety and as necessary, Sponsored Program Services.

19 BIOSECURITY

It is everyone's responsibility to ensure the security of the biological agents and materials under their control. The loss or theft of some agents and materials may have consequences greater than an accidental release in the lab. The only way to know if there has been a loss or theft is to know what you have in storage. A complete inventory of microorganisms, cells, cell lines, tissue samples, toxins and any other regulated biological materials is required for all ultralow temperature long term storage. The freezers or dewars must have access controls in place; a lock on the unit or in a locked room under your control. Units located in a shared space with public access must be locked at the freezer or dewar. Please refer to the Biological Agent Inventory Policy.

Principal Investigators (PIs) shall restrict casual traffic through laboratory areas. This is required in labs where Department of Public Health (DPH) registered materials are used. Untrained visitors must be escorted at all times by a trained member of the PI's laboratory. Visitors should be made aware of the hazards present, and precautions should be taken to ensure their safety.

If a visitor is in the lab for a short term, the PI is responsible for providing lab specific training in the lab(s). If a visitor plans to be or is still in the lab after 1 month, they must attend the EHS Safety training required by regular lab members. Ancillary personnel (e.g. repair and/or service personnel) who have not completed the appropriate UConn EHS safety training must be escorted at all times by trained lab personnel.

A visitor is anyone entering a laboratory or area posted with "Biohazard Authorized Personnel only" signage, that is not listed as a member of that lab. This applies to all UConn and non-UConn employees or affiliated individuals. If someone from another lab stores or uses items on a regular basis in a DPH registered lab, then that individual must attend the training appropriate for the DPH registered materials. A PI who is responsible for a multi-use or shared equipment laboratory is responsible for ensuring that all users have fulfilled the training requirements before allowing them unescorted access to the lab.

20 APPENDICES

Biohazard Spill Clean-up Guides

Biohazard Spill Warning Sign

Biological Exposure Incident Response