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**Standard Operating Procedure
for
Bio-safety**

REVISION

Rev No.	DCN No.	Change Summary	Release Date	DCN Initiator	Document Owner
2	DCN0654	Added: Section 2 Responsibilities; Section 11.3.3 Centrifuge Equipment Use; Section 11.3.4 Ultracentrifuge Equipment Use; Section 11.4 Autoclaves; Section 18 Ordering Procedure; Section 19 Training. Removed Table 2 and created EHS-00056-F1, removed Table 3 and created EHS-00056-F2.	5-4-11	J. Trodden	R. Segura

Prior revision history, if applicable, is available from the Document Control Office.

1. PURPOSE AND SCOPE

- 1.1 This bio-safety manual has been developed by the College of Nanoscale Science and Engineering (CNSE) to accomplish the following goals:
 - 1.1.1 Protect personnel from exposure to infectious agents
 - 1.1.2 Prevent environmental contamination
 - 1.1.3 Provide an environment for high quality research while maintaining a safe work place
 - 1.1.4 Comply with applicable federal, state and local requirements
- 1.2 The bio-safety manual provides CNSE facility wide safety guidelines, policies and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI), its success depends largely on the combined efforts of laboratory supervisors and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used.
- 1.3 In general, the handling and manipulation of biological agents and toxins, as well as recombinant DNA molecules, requires the use of various precautionary measures depending on the material(s) involved. This procedure will provide assistance in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary.

2. RESPONSIBILITIES

- 2.1 The CNSE Environmental Health and Safety (EHS) Department
 - 2.1.1 The CNSE EHS department just like the EHS department on UAlbany main campus; has the authority to require changes in unsafe operations, to assure compliance with applicable regulations and recommendations, and to maintain reasonable standards of occupational health and biological safety. Any significant problems, violations, accidents, or illnesses are reported promptly to the CNSE Security Desk, the CNSE EHS department and to the Institutional Bio-Safety Committee (IBC).
 - 2.1.2 The CNSE EHS department operates independently from the main campus' EHS department, which precludes the main campus Bio-Safety Officer (BSO) from providing oversight and authority for CNSE bio-hazardous research. All CNSE research projects that shall be conducted

on the CNSE campus must be approved and signed-off by the CNSE BSO prior to being presented to the IBC on the main campus. This system of approval will properly establish accountability for the safe conduct of research at CNSE.

2.2 Bio-Safety Officer (BSO)

2.2.1 The CNSE Bio-Safety Officer (BSO) is a member of UAlbany Institutional Bio-Safety Committee (IBC); and has the responsibility of following the directives of the IBC; and for ensuring that the policies and procedures provided in this document are complied with. The BSO operates under the direction of the CNSE EHS department and is responsible for the following:

- Oversee the policies and procedures of the program,
- Provide guidance on the requirements of the program,
- Perform assessments, review and approval of new bio-hazardous facilities,
- Perform assessments, review and approval of new bio-hazardous research and materials used in this research,
- Select and establish standard operating procedures for controls used within bio-hazardous research laboratories,
- Assist in the design and selection of new controls,
- Understand bio-safety hazards and bio-safety controls,
- Notify those working in bio-safety areas when new bio-safety hazards are introduced to their area,
- Provide bio-safety training, and
- Evaluate the effectiveness of the program on an annual basis.

2.3 Facility Engineering, laboratory owners and faculty are responsible for complying with the policies and procedure set forth by the IBC, and this program, and for supporting and enforcing both programs to ensure compliance by all personnel.

2.4 Employees, students and researchers are responsible for understanding the bio-safety hazards associated with their job task and areas in which they work, and following established policies and procedures set forth by the IBC and this program.

3. BIOLOGICAL SAFETY AND BIO-SAFETY LEVELS

- 3.1 Biological safety or bio-safety is the application of knowledge, techniques and equipment to prevent personnel, laboratory and environmental exposure to potentially infectious agents or bio-hazards. Bio-safety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine bio-hazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. It can be accomplished through the following means:
- 3.2 **Primary Containment**
- 3.2.1 Protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.
- 3.3 **Secondary Containment**
- 3.3.1 Protection of the environment external to the laboratory from exposure to infectious materials through a combination of facility design and operational practices.
- 3.4 Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Currently four Bio-safety Levels (1-4) define the level of containment necessary to protect personnel and the environment. A Bio-safety Level 1 (BL-1) is the least restrictive, while Bio-safety Levels 3 and 4 (BL-3 and BL-4) requires special containment laboratories or facilities, which are not yet available at CNSE. Since most of the research at CNSE is conducted at Bio-safety Levels 1 and 2, this manual will mainly focus on these two Bio-safety Levels. Due to the lack of engineering controls in place at CNSE to control Bio-safety Levels 3 and 4, work that may involve either of these levels is prohibited.
- 3.5 A summary of the different bio-safety level requirements (BL-1, 2 and 3) can be found in Table 1 [on page 42](#).
- 3.6 The most important element in maintaining a safe work environment is strict adherence to good micro-biological and laboratory practices and techniques. Everybody working with infectious agents or potentially infected materials must be aware of the potential risks. In addition, they must be trained and proficient in the practices and techniques required for handling such material. It is the responsibility of the Principal Investigator (PI) or person in charge of the laboratory to provide or arrange for appropriate training of all personnel. Such personnel should refer to the Bio-safety Level 1 (BL-1) and Bio-safety Level 2 (BL-2) Laboratory

Checklists [EHS-00056-F1](#) and [EHS-00056-F2](#) to determine the controls that should be put in place prior to setting up such laboratories.

4. BIOHAZARD

- 4.1 Bio-hazardous agents are biological agents and materials which are potentially hazardous to humans, animals and/or plants.
- 4.2 Bio-hazardous agents may include but are not limited to: Certain bacteria, fungi, viruses, rickettsiae, chlamydiae, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents these cells may contain; viroids, prions and other infectious agents as outlined in laws, regulations, or guidelines.
- 4.3 Biological hazards can be found in various research activities at the CNSE facility. Such biological hazards at CNSE include:
- Cells/Viruses: (bacteria, mammalian cell, virus, bacteriophage, parasite),
 - Proteins,
 - Nucleic Acid (DNA/RNA),
 - Antibiotics,
 - Biochemicals (amino acids, crosslinkers, and other chemicals).
- 4.4 Special facilities are specifically designed to address some of the infectious agent research needs for dealing with infectious agents including human, animal and plant pathogens. While animal care facilities have to address zoonotic diseases, in the human health care environment, Bloodborne pathogens pose the greatest risk. The information contained in this and other procedures will further increase our ability to maintain a safe and healthy work environment.

5. DEFINITIONS

- 5.1 **Biohazard** - Infectious or etiologic (disease causing) agents, potentially infectious materials, certain toxins and other hazardous biological materials are included in the definition of a biohazard.
- 5.2 **Bio-safety Level 1 (BL-1)** practices, safety equipment, and facilities are appropriate for projects with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans.
- 5.3 **Bio-safety Level 2 (BL-2)** practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents

present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in experiments conducted on the open bench, provided the potential for producing splashes or aerosols is low. BL-2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown.

- 5.4 **Bio-safety Level 3 (BL-3)** practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for aerosol transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a Bio-safety Cabinet (BSC) or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory. All BL-3 work is currently prohibited at the CNSE facility.
- 5.5 **Bio-safety Level 4 (BL-4)** practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents, which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Additionally, agents with a close or identical antigenic relationship to BL-4 agents should also be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BL-4. Primary hazards to personnel working with BL-4 agents are respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and to the environment. The laboratory worker's complete isolation of aerosolized infectious materials is accomplished primarily by working in a Class III BSC or a full-body, air-supplied positive-pressure personnel suit. The BL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation and waste management systems to prevent the release of viable agents to the environment. All BL-4 work is currently prohibited at the CNSE facility.

- 5.6 **Bloodborne Pathogens** - Pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, Hepatitis B virus (HBV) and Human Immunodeficiency virus (HIV).
- 5.7 **Human Subject** - An individual about whom an investigator conducting research obtains: 1) data or materials (blood, tissue, etc.) through intervention or interaction with the person, or, 2) identifiable information." (Public officials elected or in non-elected, decision-making positions, are not considered human subjects under these regulations).
- 5.8 **Research** - Formal investigation designed to develop or contribute to generalized knowledge.
- 5.9 **Universal Precautions** - A method of infection control that treats all human blood and other potentially infectious materials as capable of transmitting HIV, HBV, and other Bloodborne pathogens.

6. ROUTES OF INFECTION

- 6.1 Working in a biological research environment like CNSE, it is not unreasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:
- 6.1.1 Ingestion: Eating, drinking and smoking in the laboratory; mouth pipetting; or transfer of microorganisms to mouth by contaminated fingers or articles.
- 6.1.2 Injection: Accidental inoculation with a hypodermic needle, other sharp instrument or glass; or through cuts and scratches.
- 6.1.3 Absorption: Splashes of infectious material into the eyes or skin; or transfer of microorganisms to eyes or skin by contaminated fingers.
- 6.1.4 Inhalation: Inhalation of airborne microorganisms through the lungs.
- 6.2 In order to control the routes of infection, good laboratory practices should be adhered to, as well as, the use of primary barriers (Safety Equipment) and secondary barriers (Facility Design and Operational Practices).

7. ADMINISTRATIVE CONTROLS

7.1 Bio-hazard Warning Sign

A biohazard label is required for all areas or equipment where BL-2 or above, procedures are utilized. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, and on equipment like refrigerators, incubators, transport containers, and/or lab benches. Labels can be obtained from the EHS Office (see Figure 1).



Figure 1 - Biohazard Label

7.2 Bloodborne Pathogens Program and Exposure Control Plan

7.2.1 CNSE is committed to protecting its employees from risks associated with exposure to Bloodborne pathogens through implementation of its *Exposure Control Plan* (ECP). This plan follows the requirements established by the U. S. Occupational Safety and Health Administration (29 CFR 1910.1030). All employees at CNSE that have a reasonable anticipated risk for exposure to Bloodborne pathogens need to be included in the Bloodborne Pathogens Program. As outlined in the ECP, these employees need to be identified and provided with the appropriate means to safely conduct their individual jobs. The following principles must be followed when employees are potentially exposed to Bloodborne pathogens:

- Minimize all exposure to Bloodborne pathogens;
- Institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to Bloodborne pathogens;

- Routinely employ "Universal Precautions" when exposure to blood or potentially infectious materials is anticipated.

7.2.2 All employees covered under the ECP need to attend an initial training class on Bloodborne pathogens, as well as, an annual refresher course. Classes are offered on a regular basis at the EHS office. In addition, employees have to be provided with Hepatitis B vaccination free of charge.

7.2.3 The specific requirements and responsibilities of Principal Investigators, laboratory supervisors, employees and others are outlined in the ECP (EHS-00012). Please consult this plan for further information. Copies are available from the EHS office or online on the Intranet, <http://intranet.cnse.albany.edu>.

7.3 Training

7.3.1 Good microbiological and laboratory practices are essential for a safe work environment.

7.3.2 Training and education on these practices and procedures is essential in order to ensure adherence to this program. In addition, all personnel working in the laboratory shall receive adequate laboratory specific training from the Principal Investigator (PI) or laboratory supervisor. Training should include, at a minimum:

- Good laboratory, animal and microbiological practices as applicable,
- Site specific Safety Orientation and Advanced Safety Training classes which cover information on risks, hazards and procedures,
- Laboratory or environment specific BL-2 procedures, as applicable.

7.3.3 In addition, it is important that all personnel working at BL-2 attend the bio-safety training offered by the EHS Office or online on the Intranet, <http://intranet.cnse.albany.edu>.

7.4 Recombinant DNA Program

All research at CNSE involving recombinant DNA (rDNA), independent of the funding source, needs to be in compliance with the requirement of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*.

8. ENGINEERING CONTROLS - BIOLOGICAL SAFETY CABINETS (BSCs)

8.1 BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of Biological Safety Cabinets, designated as Class I, II and III have been developed to meet various research and clinical needs. Biological Safety Cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems. Biological Safety Cabinets must not be confused with other laminar flow devices or “clean benches”; in particular, horizontal flow cabinets which direct air towards the operator and should never be used for handling infectious, toxic or sensitizing materials.

8.2 Laboratory personnel must be trained in the correct use and maintenance of Biological Safety Cabinets to ensure that personnel and product protection (where applicable) are maintained. Before selecting any bio-safety cabinet for purchase, contact the EHS Office for a work specific assessment and selection criteria.

8.3 **Class I Biological Safety Cabinet**

This is a ventilated cabinet for personnel protection with an un-recirculated inward airflow away from the operator. This unit is fitted with a HEPA filter to protect the environment from discharged agents. A Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection (e.g., sterility).

8.4 **Class II Biological Safety Cabinet**

This is a ventilated cabinet for personnel, product and environmental protection which provides inward airflow and HEPA-filtered supply and exhaust air. The Class II cabinet has four designs depending on how much air is re-circulated and/or exhausted; and if the BSC is hard-ducted to the ventilation system or not. Class II cabinets may be of use with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radionuclides; however, care must be exercised in selecting the correct Class II cabinet design for these purposes.

8.5 **Class III Biological Safety Cabinet**

A Class III cabinet is a totally enclosed ventilated cabinet which is gas-tight, and maintained under negative air pressure (0.5 inches water gauge). The supply air is HEPA-filtered and the exhaust air has two HEPA filters in series. Work is performed in the cabinet by the use of attached rubber gloves.

8.6 Biological Safety Cabinets, when properly used in research and teaching activities involving the manipulation of bio-hazardous agents, are effective in containing and controlling particulates and aerosols; and complement good laboratory practices and procedures. The correct location, installation, and certification of the Biological Safety Cabinet are critical to its performance in containing infectious aerosols. All BSCs used for RG-2 or 3 and rDNA research must be inspected annually; and certified by trained and accredited service personnel. Inspection and re-certification is mandatory if the cabinet is relocated; or after major repairs, filter changes etc. To request service or certification contact the EHS Office.

8.7 **Safe and Effective Use of Bio-safety Cabinets**

8.7.1 General:

- Make sure the BSC is certified when it is installed or after it is moved, or repaired and annually thereafter. Check the magnehelic gauge or electronic controls regularly for any indication of a problem.
- Understand how your cabinet works (attend a BSC Safety Seminar).
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet; people walking rapidly behind you; and open lab doors, may disrupt the airflow pattern and reduce the effectiveness of the BSC.
- Plan your work.
- Minimize the storage of materials in and around the BSC.
- Always leave the BSC running.

8.7.2 Operation:

- Before using, wipe work surface with 70% alcohol or any other disinfectant suitable for the agent(s) in use. Wipe off each item you need for your procedures before placing it inside cabinet.
- DO NOT place any objects over the front air intake grille. DO NOT block the rear exhaust grille.

- Segregate contaminated and clean items. Work from “clean to dirty.”
- Place a pan with disinfectant and/or sharps container inside the BSC for pipette discard. DO NOT use vertical pipette discard canisters on the floor outside the cabinet.
- It is not necessary to flame items. This creates turbulence in airflow and will compromise sterility; heat buildup may damage the HEPA filter and release of gas may result in explosion.
- Move arms slowly when removing or introducing new items into the BSC.
- If you use a piece of equipment that creates air turbulence in the BSC (such as a microcentrifuge or blender), place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
- Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.
- Clean up spills in the cabinet immediately. Wait 10 minutes before resuming work.
- When work is finished, remove all materials and wipe all interior surfaces with 70% alcohol or any other disinfectant suitable for the agent(s) in use.
- Remove lab coat, gloves and other Personal Protective Equipment (PPE) and wash hands thoroughly before leaving the laboratory. Lab coats should not be laundered at home.

9. ENGINEERING CONTROLS - SAFETY EQUIPMENT

9.1 Safety Showers

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are outlined in ANSI Standard Z136.1.

9.2 Eyewash Stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored; and where employees perform tasks that might result in splashes of potentially infectious materials.

9.3 **Ventilation Controls**

9.3.1 Ventilation Controls are those controls intended to minimize employee exposure to hazardous chemicals and infectious or toxic substances by removing air contaminants from the work site.

9.3.2 There are two main types of ventilation controls:

- 1) General (Dilution) Exhaust: a room or building-wide system which brings in air from outside and ventilates within. Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work. General exhaust systems are inadequate for BL-3 work.
- 2) Local Exhaust or Filtration: a ventilated, enclosed work space intended to capture, contain and exhaust or filter harmful or dangerous fumes, vapors and particulate matter. In the case of hazardous chemicals, this includes a fume hood. In the case of infectious agents, BSCs should be used.

10. **PERSONAL PROTECTIVE EQUIPMENT (PPE)**

10.1 PPE is used to protect personnel from contact with hazardous materials and infectious agents.

10.2 Appropriate clothing may also protect the experiment from contamination. Personal protective devices and safety equipment must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. The following PPE is recommended for regular use.

10.3 **Face Protection**

Splash goggles or safety glasses with solid side shields in combination with face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face.

10.4 **Laboratory Clothing**

This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes

contaminated. At a minimum, a laboratory coat should be worn in all laboratories working at a BL-2.

10.5 **Gloves**

10.5.1 Gloves must be selected based on the hazards involved and the activity to be conducted.

10.5.2 Chemical resistant gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves like nitrile gloves; however protection from contact with toxic or corrosive chemicals may also be required.

10.6 **Respirators**

10.6.1 For certain protocols and projects, additional PPE like respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required.

10.6.2 Personnel who require respiratory protection must contact the EHS Department for assistance in selection of proper equipment and training in its usage. Personnel wearing respirators need to be included in the CNSE *Respiratory Protection Program* (see EHS-00015).

11. **RECOMMENDED WORK PRACTICES**

11.1 **Pipettes and Pipetting Aids**

11.1.1 Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of bio-hazardous or toxic fluids to a bio-safety cabinet, if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench. Use the following precautions:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette.
- Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.

- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a bio-safety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet while in use.

11.2 **Syringes and Needles**

11.2.1 Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. The PI should create a list of authorized syringe and needle users; and keep all syringes and needles in a locked cabinet.

11.2.2 When using syringes and needles with biohazardous or potentially infectious agents:

- Work in a bio-safety cabinet whenever possible.
- Wear gloves.
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid and bubbles from the syringe vertically into a cotton pad moistened with a disinfectant.

11.3 **Centrifuge Equipment**

11.3.1 Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions including safety precautions should be prominently posted on the unit.

11.3.2 Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and re-suspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging bio-hazardous material, the following procedures should be followed.

11.3.3 Centrifuge Equipment Use

- Sealed tubes and safety buckets that seal with O-rings shall be used. Before use, tubes, O-rings and buckets shall be inspected for cracks, chips, erosions, bits of broken glass, etc. Aluminum foil shall not be used to cap centrifuge tubes, as it may detach or rupture during centrifugation.
- Centrifuge tubes, rotors and accessories shall be filled and opened in a BSC. Overfilling of centrifuge tubes shall be avoided so that closures do not become wet. After tubes are filled and sealed, they shall be wiped down with disinfectant.
- Disinfectant shall be added to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
- Buckets, tubes and rotors shall be balanced properly before centrifugation.
- Supernatant shall not be decanted or poured off. A vacuum system shall be used with appropriate in-line reservoirs and filters.
- A BSC shall be used when re-suspending sedimented material. A swirling rotary motion shall be used rather than shaking. If shaking is necessary, aerosols shall be permitted to settle before opening the tube by waiting a few minutes before opening.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions shall be taken to filter the exhaust air from vacuum lines; to avoid metal fatiguing resulting in disintegration of rotors; and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations shall be meticulously followed to avoid metal fatigue, distortion and corrosion.
- The use of celluloid (cellulose nitrate) tubes with bio-hazardous materials should be avoided as celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They become distorted upon boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants shall be used for decontamination.

11.3.4 Ultracentrifuge Equipment Use

- G-force rated (RCF) centrifugation tubes shall be used. Only tube material that is compatible with the solutions to be centrifuged shall be used. No other tubes than those recommended by the manufacturer for each specific rotor shall be used. If the tubes can be sealed, screwing the caps with an O-ring seal is recommended.
- Before use, tubes, O-rings and rotors shall be inspected for cracks, chips, erosion, bits of broken glass, etc. If there is evidence of cracks in tubes/rotors, the centrifuge shall not be used. Aluminum foil shall not be used to cap centrifuge tubes as it may detach or rupture during centrifugation.
- Centrifuge tubes shall not be overfilled. Manufacturer's recommendations regarding fill levels of the centrifuge tubes shall be followed. After tubes are filled and sealed they shall be wiped down with disinfectant.
- Buckets, tubes and rotors shall be properly balanced before centrifugation. Tubes/rotor shall be balanced to be within 0.01g (10mg).
- To minimize exposure to aerosols, centrifuge tubes shall be opened in a Bio-safety Cabinet with the open end pointed away from you.
- Supernatant shall not be decanted or poured off. An aspirator/pipette with appropriate in-line reservoirs and filters shall be used.
- When re-suspending sedimented material, a swirling rotary motion shall be used rather than shaking. If shaking is necessary, aerosols shall be permitted to settle before opening the tube by waiting a few minutes before opening.
- High-speed centrifuges pose additional hazards. Precautions shall be taken to filter the exhaust air from centrifuge vacuum lines (exhaust air-filter or vent to vacuum line). Manufacturer's recommendations shall be meticulously followed to avoid metal fatigue, distortion and corrosion.
- If using biological material in the centrifuge, the rotor shall be removed and the rotor and centrifuge housing shall be wiped with disinfectant when the operation is complete.

11.4 Autoclaves

11.4.1 Operational Requirements

11.4.1.1 Each lab seeking to operate an autoclave shall develop an operation plan, and shall submit such plan to EHS for review and approval prior to using the autoclave to treat regulated medical waste.

11.4.1.2 Any change in procedures related to the operation of the autoclave shall be reflected in a modified plan, which shall be dated with the revision and submitted to the EHS department for review and approval.

11.4.2 Operation Procedure

- The water reservoir shall be full. If the reservoir is low, refill with only dH₂O (do not use regular tap water).
- Items that require autoclaving shall be placed on the autoclavable tray and in the autoclave.
 - 1) Material to be autoclaved shall be placed in an autoclavable tray.
 - 2) The autoclaved items must have indicator tape.
 - 3) Liquids shall be separated from solids, and then autoclaved separately.
 - 4) All lids on all bottles shall be adjusted to be loose. Crowding large number of items on the tray shall be avoided as this then allows for all items to be autoclaved to reach the appropriate temperature
 - 5) One must allow sufficient air/steam circulation.
- The door shall be closed so that it latches shut and the door clamp shall be tightened over the latch.
- The program to be used shall be chosen. Determine program from the program list (liquid, wrapped items, etc.).
- If unsure, the liquids setting shall be used (this is the longest program). Items that will be dry (tubes, sterile sticks, etc.) should be run on one of the cycles that includes a drying step.
- BSL1 waste shall be taped shut with autoclave indicator tape and shall be run on the "Liquids" program.
- The start button shall then be pressed.

- Confirm that the program has started and that the temperature is rising.
- The autoclave will beep when the cycle is finished – press the STOP button.
- The chamber pressure should be 0 psi
- The temp should be <100°C
- Open door.
- Remove contents using gloves.
- Tighten all caps once contents have cooled to room temperature.
- Wipe inside of autoclave with a paper towel to remove excess moisture/liquid that collects in the chamber.

11.4.3 Autoclave Failure Procedure

11.4.3.1 Whenever an autoclave fails to operate in accordance with pre-established operating parameters, the lab shall:

- Discontinue use of the autoclave, using emergency shutdown procedures if appropriate, until corrective action has been taken and validation testing has verified that effective treatment can resume
- Handle as untreated all regulated medical waste processed by the system since the last previous run under documented compliance with such requirements
- Document the failure, including date and autoclave identifier
- Document the lab response, including corrective action

11.4.3.2 Whenever a lab has reason to believe untreated BSL2 waste certified as treated waste has left the facility, notify the waste transporter as soon as practicable, and notify the DOH within seventy-two (72) hours of the waste's leaving the facility.

11.4.4 Validation Testing

11.4.4.1 Testing shall be performed on any autoclave that is used to treat BSL2 level waste.

- 11.4.4.2 Testing shall be performed under conditions that include load configuration, composition, and volume that simulate conditions anticipated during actual waste treatment.
- 11.4.4.3 *Geobacillus stearothermophilus* spores will be used at a minimum concentration of 6 log₁₀ spores per indicator unit and will be placed in the center of the load or otherwise coldest point in the autoclave chamber as indicated by the manufacturer.
- 11.4.4.4 Alternative and/or supplemental indicators will be used, as necessary, to demonstrate effectiveness of treatment.
- 11.4.4.5 Effective treatment shall be demonstrated by a 4 log₁₀ reduction in viable *Geobacillus stearothermophilus* spore concentration or other measure of effectiveness for alternative and/or supplemental indicators.
- 11.4.4.6 Any autoclave that fails to meet the criteria for effective treatment upon validation shall not be used to treat bio-hazardous materials.
- 11.4.5 Record Keeping
- 11.4.5.1 Each lab shall retain for three (3) years records of: validation testing and challenge testing, including protocols and test results; routine system's monitoring; and, where applicable, DOH approval as an alternative treatment technology.
- 11.4.5.2 Each lab shall document each employee's participation in training and/or retraining in autoclave operations, and shall retain such records for three (3) years.
- 11.4.5.3 Documentation of corrective action, including that required by Section 11.4.5, of this Subpart, shall be retained for three (3) years.
- 11.4.5.4 Documentation of any modification to an approved operation plan, regardless of whether the modification is subject to DOH approval pursuant to this section, shall be retained three (3) years from discontinuance of the modified plan.
- 11.4.5.5 Documentation of the residence time, pressure and temperature of each load treated shall be retained for three (3) years.
- 11.4.5.6 All records required pursuant to this section shall be made available for inspection and copying by the EHS department and the DOH.

11.5 Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers

The use of any of these devices results in considerable aerosol production. Blending, cell disrupting and grinding equipment should be used in a BSC when working with bio-hazardous materials.

11.5.1 Safety Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. If blender rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with bio-hazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

11.5.2 Lyophilizers and Ampoules

11.5.2.1 Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

11.5.2.2 Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as bio-hazardous waste.

11.5.2.3 Ampoules used to store bio-hazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized and are fitted with polyethylene caps

with silicone washers. Heat sealable polypropylene tubes are also available.

11.5.3 Loop Sterilizers and Bunsen Burners

11.5.3.1 Sterilization of inoculating loops or needles in an open flame generates small particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended.

11.5.3.2 Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

11.6 Laundry

11.6.1 All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible; and needs to be collected in special hampers (labeled or color coded) or in biohazard bags. Laundry must be subcontracted by the owner of the bio-safety lab. Appropriate PPE must be worn by employees who handle contaminated laundry.

11.7 Housekeeping

11.7.1 Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

11.7.2 Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes - old and unused chemicals should be disposed of promptly and properly. Refer to CNSE Waste Management Plan EHS-00009 for more information.

- Providing a workplace that is free of physical hazards - aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety: especially as it relates to the use of extension cords; proper grounding of equipment; and avoidance of the creation of electrical hazards in wet areas.
- Complying with SUNY at Albany Chemical Hygiene Plan.
- All laboratory equipment needs to be decontaminated before being released for repair or maintenance.

12. BIOHAZARD SPILL CLEAN-UP PROCEDURES

12.1 Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards should have a basic biological spill kit ready to use at all times. For most instances, the basic kit can be assembled with materials already used in the laboratory. Although it is preferable to have the content of the spill kit in one location, as long as the materials are easily accessible to everyone in the lab, prior assembly might not be necessary. However, ready assembled spill kits are available through EHS department.

12.2 Basic Biological Spill Kit:

- Disinfectant (e.g., bleach 1:10 dilution, prepared fresh)
- Absorbent Material (e.g., paper towels)
- Waste Container (e.g., biohazard bags, sharps containers)
- Personal Protective Equipment (e.g., lab coat, gloves, eye and face protection)
- Mechanical Tools (e.g., forceps, dustpan and broom)
- The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency situation, stay calm, call (518) 437-8600 or 78600.

12.3 Spill Inside the Laboratory (BL-2)

12.3.1 Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further processing. Don a disposable gown or lab coat, safety goggles and gloves.

12.3.2 Have a complete biological spill kit ready to go before you start the clean-up.

12.3.3 Initiate cleanup with disinfectant as follows:

- Cover spill with paper towels or other absorbent material containing disinfectant.
- Encircle the spill with disinfectant (if feasible and necessary), being careful to minimize aerosolization.
- Decontaminate and remove all items within spill area.
- Remove broken glassware with forceps or broom and dustpan and dispose in sharps container. Do not pick up any contaminated sharp object with your hands.
- Remove paper towels and any other absorbent material; and dispose in biohazard bags.
- Apply disinfectant to the spill area and allow for at least 10 minutes contact time to ensure germicidal action of disinfectant.
- Remove disinfectant with paper towels or other absorbent material; and dispose of in biohazard bag.
- Wipe off any residual spilled material and reapply disinfectant before final clean-up.
- Wipe equipment with equipment compatible disinfectant (e.g., non-corrosive). Rinse with water, if necessary.
- Place disposable contaminated spill materials in biohazard bags for disposal.
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving.
- Reopen area to general use only after spill clean-up and decontamination is complete.
- Inform all personnel and laboratory supervisor about the spill and successful clean-up, as soon as possible.

12.4 Spill Inside the Biological Safety Cabinet (BL-2)

Have a complete biological spill kit ready to go before you start the clean-up.

- Wear lab coat, safety goggles and gloves during clean-up.
- Allow cabinet to run during clean-up.
- Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 10 minutes contact time.
- Wipe up spillage and disinfectant with disposable paper towels.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.
- Discard contaminated disposable materials in biohazard bag(s) and place in bio-waste container.
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving and further clean-up.
- Expose non-autoclavable materials to disinfectant, 10 minutes contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for further processing by laundry.
- Run cabinet at least 10 minutes after clean-up and before resuming work.
- Inform all users of the BSC, as well as, the laboratory supervisor about the spill and successful clean-up as soon as possible.

12.5 Spill Inside a Centrifuge

Have a complete biological spill kit ready to go before you start the clean-up.

- Clear area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up the spill.
- Wear a lab coat, safety goggles and gloves during clean-up.
- Remove rotors and buckets to the nearest Biological Safety Cabinet.
- Thoroughly disinfect inside of centrifuge.
- Remove contaminated debris after disinfection, place in appropriate biohazardous waste container(s) and autoclave before disposal.

- 12.6 Spill Outside the Laboratory, During Transport on Site
- 12.7 Always transport biohazardous materials in an unbreakable well-sealed primary container placed inside a leak proof, closed and unbreakable secondary container, labeled with the biohazard symbol (plastic cooler, bio-specimen pack, etc.).
- 12.8 Should a spill of BSL-2 material occur in the public, call the emergency number (518) 437-8600 or 78600 immediately. Do not attempt to clean up the spill without the proper personal protective equipment and spill clean-up material.
- 12.9 Should the spill occur inside a lab and you are unsure of what to do call the emergency number (518) 437-8600 or 78600 immediately.

13. DECONTAMINATION

13.1 Methods of Decontamination

- 13.1.1 Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means, that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present.
- 13.1.2 Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of reusable containers such as glass flasks, bottles, test tubes, pipettes, etc are done by sterilization in an autoclave.
- 13.1.3 In order to select the proper method and tools, it is important to consider, for example, the following aspects:
- Type of biohazardous agents, concentration and potential for exposure;
 - Physical and chemical hazards to products, materials, environment and personnel.
- 13.1.4 Physical and chemical means of decontamination fall into four main categories:
- Heat,
 - Liquid Chemicals,

- Vapors and Gases, and
- Radiation.

13.1.5 Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time. The following paragraphs will focus on some of these methods.

13.2 Heat

13.2.1 In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in overall shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 121-132°C (250-270°F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170°C (320-338°F) for periods of 2 to 4 hours.

13.3 Liquid Chemicals Used as Disinfectants

13.3.1 The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:

- Nature of surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to be effective.
- Number of microorganism present - Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms - Microbial agents can be classified according to increasing resistance to disinfectants and heat.
- Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

13.3.2 There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are used at the CNSE facility are discussed below:

13.3.3 Alcohols:

13.3.3.1 Ethyl or isopropyl alcohol in concentration of 70% to 90% are good general-use disinfectants.

13.3.3.2 However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores. Concentrations above 90% are less effective.

13.3.4 Chlorine:

13.3.4.1 Chlorine-containing solutions have broad spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Diluted solutions may be kept for extended periods if kept in a closed container and protected from light. However, it is recommended to use freshly prepared solutions for spill clean-up purposes. Chlorine containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive so they can not be used on surfaces that can corrode (e.g. painted surfaces, metals, etc). Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilants, since they inactivate bacterial spores.

14. **BIO-HAZARDOUS WASTE**

14.1 At CNSE, the term Bio-hazardous Waste is used to describe different types of waste that might include infectious agents. Currently, the following waste categories are all considered to be Bio-hazardous Waste:

14.2 **Regulated Medical Waste:** Defined by the Environmental Conservation Law, Part 360 and Part 364 regulations as any solid waste which is generated in the diagnosis, treatment (e.g., provision of medical services), or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals. BSL-1 materials do not fall under the regulated medical waste category. Regulated medical waste includes:

- Cultures and stocks of infectious agents and associated biologicals, including laboratory waste, biological production waste, discarded live and attenuated vaccines, culture dishes, and related devices.
- Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or materials stained with blood or body fluids.

- Pathological waste: defined as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
- Sharps: Defined as needles, syringes, scalpels, and intravenous tubing with needles attached, regardless of whether they are contaminated or not.
- Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.

14.3 **Regulated Waste** as defined by the *Occupational Safety and Health Act on Bloodborne Infectious Diseases* including:

- Liquid or semi-liquid blood or other potentially infectious materials;
- Contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state, if compressed;
- Items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling;
- Contaminated sharps which includes any contaminated object that can penetrate the skin;
- Pathological and microbiological wastes containing blood or other potentially infectious materials.

14.4 **Laboratory Waste and Regulated Waste** as defined in the *Guidelines for Research Involving Recombinant DNA Molecules* (NIH) and the *CDC/NIH Bio-safety in Microbiological and Biomedical Laboratories*.

14.4.1 The CDC/NIH Bio-safety Guidelines cover contaminated waste that is potentially infectious or hazardous for humans and animals. The same is true for the NIH Guidelines on recombinant DNA which also cover contaminated waste potentially infectious or hazardous for plants.

14.5 **General Labeling, Packaging and Disposal Procedures**

14.5.1 Currently, biohazardous waste BSL-2 or greater shall not be decontaminated before leaving CNSE. Most of the waste will be autoclaved prior to disposal by an outside vendor, while some waste will be incinerated. The responsibility for proper disposal of bio-hazardous waste lies with the laboratory and department producing the waste.

14.5.2 All biohazardous waste needs to be packaged, contained and located in a way that protects and prevents the waste from release, at any time, at the

producing facility prior to ultimate disposal. If storage is necessary, putrefaction and the release of infectious agents in the air must be prevented.

- 14.5.3 No bio-hazardous waste can be stored for more than 90 days at CNSE.
- 14.5.4 If not stated otherwise, most bio-hazardous waste will be disposed of in biohazard bags. Currently, CNSE requires the use of red biohazard bags that include the biohazard symbol. Bags that meet these requirements are available in various sizes at the EHS department.

15. WASTE SPECIFIC PROCEDURES FOR BL-1 AND BL-2

15.1 Cultures, Stocks and Related Materials

15.1.1 BSL-1 cultures and stocks of infectious agents and associated biologicals (as defined above), shall be placed in autoclave biohazard bags; decontaminated by autoclaving in the on-site laboratory; and then placed in the regular trash to be picked up by the on-site waste hauler.

15.1.2 BSL-2 or greater cultures and stocks of infectious agents and associated biologicals (as defined above), shall be placed in biohazard bags; picked up by the chemical and gas handlers; and decontaminated by autoclaving by an outside vendor. Double or triple bagging may be required to avoid rupture or puncture of the bags.

15.2 Liquid Bio-hazardous Waste, Blood and Blood Products

15.2.1 BSL-1 liquid waste will be disposed of by flushing down the laboratory sink drain, as this waste does not require decontamination.

15.2.2 BSL-2 liquid waste will be decontaminated immediately after production by addition of bleach (sodium hypochlorite) to create a final concentration of 10% bleach by volume. After the addition of the bleach to the waste, the solution will be allowed to sit for an incubation time of 45 minutes; after which the pH of the solution will be tested by using pH testing strips. The target pH for the solution will be between pH 6 and pH 8. If the pH of the solution is within this range, it will be disposed of by flushing down the laboratory sink drain. If the pH is significantly higher than pH 8, it will be neutralized by addition of 1M hydrochloric acid (HCl) to achieve a pH within the acceptable range. If the pH is lower than pH 6, 1M sodium hydroxide (NaOH) will be added to achieve a pH within the acceptable range.

15.2.3 However, due to coagulation, flushing of large quantities of blood down the sanitary drain is impractical. Contact the EHS department for additional information on disposal of large volumes of blood.

15.3 **Sharps**

15.3.1 All sharps must be placed in a rigid, puncture resistant, closable and leak-proof container, which is labeled with the word “Sharps” and the biohazard symbol. CNSE approved sharps containers are available through the EHS office. Food containers (e.g., empty coffee cans) are not permissible as sharps containers. All sharps must be handled with extreme caution. The clipping, breaking, and recapping of needles is not recommended. Sharps containers should not be filled more than 2/3. After use, the container needs to be closed and labeled with a CNSE Hazardous Materials Label. To comply with the 90-day storage limit, contact the chemical and gas handlers for pick-up as soon as possible. Never place any type of sharps in the garbage.

15.4 **Contaminated Solid Waste**

15.4.1 Contaminated solid waste includes cloth, plastic and paper items that have been exposed to agents infectious or hazardous to humans, animals, or plants. These contaminated items, if BSL-1 shall be placed in autoclave biohazard bags and decontaminated by autoclaving in the on-site laboratory; and then placed in the regular trash to be picked up by the on-site waste hauler. If these contaminated items are BSL-2 or greater they shall be placed in a biohazard bag and decontaminated by autoclaving by an outside vendor. Double or triple bagging may be required to avoid rupture or puncture of the bags. Contaminated pasteur pipettes are considered sharps and need to be disposed of in a sharps container.

16. **RESTRICTED AGENTS (CDC)**

16.1 In order to prohibit the unlawful use and distribution of certain infectious organisms and toxins, the Centers for Disease Control and Prevention (CDC) have established certain restrictions. All agents included in the following list must be registered with the EHS office. In order to receive any of these agents, all acquisition requests need to be handled by the EHS office. This includes transfers in-between workgroups, universities or laboratories, purchasing from chemical manufactures, as well as, any other shipment or acquisition. Laboratories and Principal Investigators need to be approved prior to receiving and working with these agents. The EHS office is required to track these agents from the time of acquisition to final disposal. Please contact the EHS Office for more information. Some of the agents listed below are classified as BL-3 or BL-4 and will require containment procedures and facilities not available at CNSE.

16.2 Viruses

- Crimean-Congo Haemorrhagic Fever virus
- Eastern Equine Encephalitis virus
- Ebola viruses
- Equine Morbillivirus
- Lassa Fever virus
- Marburg virus
- Rift Valley Fever virus
- South American Haemorrhagic Fever Viruses (Junin, Machupo, Sabia, Flexal, Guanarito)
- Tick-borne Encephalitis Complex Viruses
- Variola Major Virus (Smallpox Virus)
- Venezuelan Equine Encephalitis virus
- Viruses Causing Hantavirus Pulmonary Syndrome
- Yellow Fever virus
- Exemptions: Vaccine strains of viral agents (Junin Virus strain candid #1, Rift Valley fever virus strain MP-12, Venezuelan Equine encephalitis virus strain TC-83, Yellow fever virus strain 17-D) are exempt.

16.3 Bacteria

- Bacillus Anthracis
- Brucella Abortus, B. Melitensis, B. Suis
- Burkholderia (Pseudomonas) Mallei
- Burkholderia (Pseudomonas) Pseudomallei
- Clostridium Botulinum
- Francisella Tularensis

- Yersinia Pestis
- Exemptions: vaccine strains as described in Title 9 CFR, Part 78.1 are exempt.

16.4 **Rickettsiae**

- Coxiella Burnetii
- Rickettsia Prowazekii
- Rickettsia Rickettsii

16.5 **Fungi**

- Coccidioides immitis

16.6 **Toxins**

- Abrin
- Aflatoxins
- Botulinum toxins
- Clostridium Perfringens Epsilon toxin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Shigatoxin
- Staphylococcal enterotoxins
- Tetrodotoxin
- T-2 toxin

16.6.1 Exemptions: Toxins for medical use, inactivated for use as vaccines, or toxin preparations for biomedical research use at an LD₅₀ for vertebrates of more than 100 nanograms per kilogram body weight are exempt. National standard toxins required for biologic potency testing, as described in 9 CFR Part 113, are exempt.

16.7 Recombinant Organisms / Molecules

16.7.1 Genetically modified microorganisms or genetic elements from organisms listed above, shown to produce or encode for a factor associated with a disease.

16.7.2 Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins listed above, or their toxic subunits.

16.8 Other Restrictions

16.9 The deliberate transfer of a drug resistance trait to microorganisms listed in this Appendix that are not known to acquire the trait naturally is prohibited by NIH *Guidelines for Research Involving Recombinant DNA Molecules*, if such acquisition could compromise the use of the drug to control these disease agents in humans or veterinary medicine.

17. SAFE HANDLING OF SOLIDS/POWDERS

17.1 This guidance outlines acceptable engineered containment, ventilation control, and respiratory protective equipment requirements necessary to keep exposure levels to hazardous solids/powders within established safe exposure levels.

17.2 Definitions

17.2.1 **Routes of Exposure** - Exposure to a substance may be hazardous by inhalation, ingestion, absorption through the mucous membranes, or by contact with the skin (allergic response or chemical burns). Exposure to airborne dust or vapors generally involves both an inhalation and an ingestion risk. The body's natural defense mechanisms will stop some fraction of dust or vapor from entering the lungs. This fraction however, will largely be ingested.

17.2.2 **Respiratory Hazard (RH) Category** - For chemical solids used at CNSE at where data available is inadequate to accurately establish a specific permitted exposure level, they shall be assigned one of the six respiratory hazard categorization bands: RH1 through RH6.

- **RH1:** A substance in this hazard category will have a low respiratory hazard. Any health effects will be minor and easily reversible and generally will occur at very high exposure levels. Exposure levels above the boundary level are met within the dustiest of production operations. Conventional local exhaust ventilation is usually adequate to control exposure to a safe level. Examples: nuisance dusts and radiopaques.

- **RH2:** A substance in this hazard category will have a moderate respiratory hazard. Inhalation of RH2 substances may cause respiratory irritation or acute or long-term health effects. Exposure levels above the lower boundary level are common in the production operations. Conventional local exhaust ventilation and respiratory protection are usually adequate to control exposures to a safe level.
- **RH3:** A substance in this category presents a serious respiratory hazard. Inhalation of an RH3 substance may cause significant respiratory irritation, toxic systemic effects, pharmacological effects or cause long-term damage to health. Exposure levels considerably above the lower boundary level are common in the production operations. Specialized local exhaust ventilation or laminar flow booths and partial containment together with full-face respiratory protection equipment is required to control exposure.
- **RH4:** A substance in this category presents a very serious respiratory hazard. Inhalation of an RH4 substance may cause severe respiratory irritation, sensitization, very toxic systemic effects, very potent pharmacological effects and serious long-term damage to health. Exposure levels considerably above the lower boundary level will be found wherever open handling of the dry material is carried out. Sophisticated control systems are required to safely handle RH4 materials in production, pilot and kilo lab quantities. Air-supplied respiratory protection equipment is required for handling operations, along with a strategic mix of isolation, containment, laminar flow booths and appropriate PPE.
- **RH5:** A substance with an extremely serious respiratory hazard. Exposure to an RH5 substance will cause extremely serious respiratory sensitization, very potent pharmacological effects, very severe systemic toxic effects and very serious long-term damage to health. These effects will occur at very low levels. The seriousness of the effect and the low level at which they can occur will require containment facilities and handling systems during piloting and production.
- **RH6:** Health effects are so severe and occur at such low levels, RH6 substances require complete isolation using state of the art containment facilities and handling systems on all scales of operation.

NOTE: As the respiratory hazard category increases from RH1 through RH4 and above, so the risk associated with the spread of contamination increases relative to that of airborne dust inhalation. The precautions

required to control contamination of RH4 and higher categories, become increasingly sophisticated.

- 17.2.3 **Dermal and Eye Hazard Categorization** - A separate categorization based on dermal and eye hazards are not used in the CNSE system. It should be recognized that the relative importance of dermal/eye contamination as a route of entry, increases with the hazard category of a substance. The ingestion of a few particles of an RH5 or RH6 contamination could easily exceed the maximum permitted dose per day. Hence the need for sophisticated (both administrative and engineering) precautions to prevent the spread of contamination during transfer operations.
- 17.2.4 **Potent Compounds** – Compounds having an RH rating RH4, RH5, or RH6.
- 17.2.5 **Exposure Risk Assessment** - assessing the risk factors and recommending the controls appropriate to the task and material involved.
- 17.2.6 **HEPA** – High Efficiency Particulate Air
- 17.2.7 **LEV** – Local Exhaust Ventilation
- 17.2.8 **RPE** – Respiratory Protective Equipment
- 17.3 **Procedure**
- 17.3.1 This guide is largely concerned with indicating the engineering controls required to keep exposures to hazardous chemicals below the relevant concentration. However appropriate engineering controls will not in themselves ensure that exposures are not exceeded.
- 17.3.2 They need to be a part of an overall control strategy, which includes all the elements that form part of good occupational health practice. Such as:
- Correct Design of Facilities
 - Exposure Risk Assessment Program
 - Access Control
 - Correct Selection and Maintenance of PPE
 - Planned Maintenance of Control Equipment (and PPE)
 - Facilities for washing, decontamination and disposal (or storage) of PPE

- Occupational Hygiene Training
- Careful instruction, supervision and training
- Medical Surveillance

- 17.4 Tables 4 and 5 are intended to provide generic guidance for the handling of RH1 to RH6 substances at CNSE in quantities ranging from 1 gram (1g) up to 10 kilos (10Kg). For materials that are in an unusually dusty form (air milled or micronized) more stringent precautions might be appropriate. Conversely it may be overly conservative for granular powders.
- 17.5 If the powders or solids can be prepared into a solution, then the respiratory hazard ratings, RH1 to RH6 and controls for these ratings no longer apply.
- 17.6 The method used to categorize a chemical will depend on how much data is available; and to some extent its stage in the development process. A number of governmental organizations publish permitted exposure levels. Hazard categorization for chemicals that have a published permitted exposure level may be made directly, using the table below.

<u>Respiratory Hazard (RH)</u>	<u>Dusts</u>
<u>Category</u>	<u>Exposure Level $\mu\text{g} / \text{m}^3$</u>
RH1	> 1000
RH2	100 to 1000
RH3	20 to 100
RH4	1 to 20
RH5	0.1 to 1
RH6	< 0.1

- 17.7 For hazardous substances on the market in the E.U., the law requires that they are classified as below and that appropriate symbols are used on labeling.
- Very toxic (T+)
 - Toxic (T)
 - Corrosive (C)
 - Harmful (X_n)
 - Irritant (X_i)

- 17.8 The law also requires that specified risk phrases be used to identify the nature of the hazard, see Table 5 – *E.U. Risk Classification Phrase Table* below. It is possible to make use of these phrases to categorize the substance to which they refer. Often, more than one risk phrase is used for a chemical. In such cases, separate categorizations should be carried out for each risk phrase and the most conservative should be used.

18. ORDERING PROCEDURE

18.1 Ordering Procedure for Biological materials: BSL1& BSL2

18.1.1 Search company website for “Spec Sheets” for desired BLS material.

- If after a good faith effort is made to find the sheets none are available, continue with the ordering process ensuring to add “Spec Sheets required with shipment” to the order e-mail that is sent to purchasing.
- If the specimen is being delivered from a lab, opposed to a manufacturer; request that the lab send a Spec Sheet if available.

18.1.2 Spec Sheets should be e-mailed to EHS prior to ordering. A Chemical Authorization form is not required.

- If a Spec Sheet was not obtained, an e-mail must be sent to EHS informing them of the impending order.

18.1.3 When specifying the ordering lab, list the name of the lab followed by “Bioreagent”.

- Ex: PI Name-Bioreagent

18.1.4 Orders should be placed in the PIs name; students’ names may be included as a secondary contact if need be.

18.1.5 When the order arrives, soft copies of the Spec Sheets must be sent by the requestor to EHS if they were not obtained prior to ordering.

18.2 Ordering Procedure for Biological Kits and Dyes

18.2.1 Review the Approved Chemical list to identify if the material has already been approved. (<http://intranet.cnse.albany.edu/> under the EHS Tab)

- If approved, continue to step 18.2.4.
- If not approved, continue to step 18.2.2.

- 18.2.2** Search the company website for the MSDS sheets.
- All MSDS sheets must be obtained. If the sheets for the kit components are listed separately, they must all be obtained.
 - If after a good faith effort is made to find the MSDS sheets none are available, continue with the ordering process ensuring to add “MSDS required with shipment” to the order paperwork.
- 18.2.3** MSDS sheets should be e-mailed to EHS prior to ordering. A Chemical Authorization form is not required.
- If an MSDS sheet was not obtained, an e-mail must be sent to EHS informing them of the order.
- 18.2.4** When specifying the ordering lab, list the name of the lab followed by “Bioreagent”.
- Ex: PI Name-Bioreagent
- 18.2.5** Orders should be placed in the PIs name; students’ names may be included as a secondary contact if need be.
- 18.3** When the order arrives, soft copies of the MSDS sheets must be sent by the requestor to EHS if they were not obtained prior to ordering.
- 18.4** **Ordering Procedure for Chemicals**
- 18.4.1** Refer to EHS-00002 for Chemical Authorization Policy.
- 19. TRAINING**
- 19.1** Bio-safety training must be completed prior to providing a student, faculty, researcher access to a bio-safety laboratory/area. This training is provided so that the user is instructed on the proper identification of bio-safety hazards prior to first use and before they are able to protect themselves from the hazard.
- 19.2** Bio-safety protection training should be a combination of online and hands-on training. The online training should cover the bio-safety fundamentals, while hands-on training is based upon lab-specific bio-safety applications and controls.
- 19.3** Upon completion of the required on-line training a written test is completed, which contains the name or other identity of the person

trained, the date(s) of the training, and the name of the person's adviser/professor or supervisor.

- 19.4 Retraining shall be conducted when the adviser/professor or supervisor has reason to believe that any affected employee who has already been trained does not have the understanding and skill required by this program.
- 19.5 Training records are kept on file in the CNSE EHS office.

20. TABLES

- 20.1 **Table 1** - Summary of Bio-safety Levels for Infectious Agents (BL-1 to BL-3)
- 20.2 **Table 2** – Control Guidance Summary Handling 1g or less
- 20.3 **Table 3** – Control Guidance Summary for Labs Handling up to 100g
- 20.4 **Table 4** – Control Guidance Summary for Labs Handling 1-10 Kilo (Kg) Quantities
- 20.5 **Table 5** - E.U. Risk Classification Phrase Table

TABLE 1
SUMMARY OF BIO-SAFETY LEVELS FOR INFECTIOUS AGENTS (BL-1 TO BL-3)

Bio-safety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
BL-1	Not known to cause disease in healthy adults	Standard Microbiological Practices	PPE: laboratory coats, gloves, safety glasses, face protection as needed	Open bench top sink required
BL-2	Associated with human disease, hazard (exposure) = auto-inoculation, ingestion, mucous membrane exposure	BL-1 practice plus: Limited access; biohazard warning signs; "Sharps" precautions; bio-safety manual defining any needed waste decontamination or medical surveillance policies	Class I or II Biological Safety Cabinets (BSCs) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats, gloves, safety glasses, face protection as needed	BL-1 plus: Autoclave available
BL-3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BL-2 practice plus: Controlled access; decontamination of all waste; decontamination of lab clothing before laundering; baseline serum	Class I or II BSCs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing, gloves, safety glasses, face protection, and respiratory protection as needed	BL-2 plus: Physical separation from access corridors; self-closing, double door access; exhausted air not re-circulated, negative airflow into laboratory

TABLE 2
CONTROL GUIDANCE SUMMARY HANDLING 1g OR LESS

Hazard Category	Controls
RH1 to RH3	May be handled on an open bench provided appropriate precautions are taken against spillage, personnel contamination and inhalation of vapor.
RH4	Must be handled within enclosed fume hood or Class II Biological Safety Cabinet. Special PPE and decontamination procedures required.
RH5 and RH6	Must be handled within glove boxes, downdraught laminar flow cabinet equipped with HEPA filtration or fume hood modified with hand ports and gloves.

NOTE 1: For dusty operations the controls should be upgraded to those appropriate to the next higher hazard category.

NOTE 2: For substances in solution the controls should be downgraded to that appropriate to the next lowest hazard category – provided appropriate precautions are taken to contain/control spillage.

TABLE 3
CONTROL GUIDANCE SUMMARY FOR LABS HANDLING UP TO 100g

Hazard Category	Controls
RH1 and RH2	May be handled on open-air bench provided appropriate precautions are taken against spillage, personnel contamination and inhalation of vapor.
RH3	Must be handled inside enclosed fume hood.
RH4	Low exposure risk operations may be handled inside enclosed fume hood with special PPE and decontamination procedures required. High exposure risk operations require the same controls required for RH5 handling (see below).
RH5	Must be handled within glove boxes, downdraught laminar flow cabinet equipped with HEPA filtration or fume hood fitted with hand ports and gloves. Special decontamination procedures required.
RH6	Handle in total containment equipment only.

NOTE 1: For dusty operations the controls should be upgraded to those appropriate to the next higher hazard category.

NOTE 2: For non-dusty operations, substances in solution or in granular form the controls could be relaxed to those appropriate to the next lowest hazard category.

NOTE 3: For highly dusty operations an exposure risk assessment should be carried out to determine the most cost effective, safe way of controlling the operation.

TABLE 4
CONTROL GUIDANCE SUMMARY FOR LABS HANDLING 1-10 KILO QUANTITIES

Hazard Category	Low Exposure Level	Medium Exposure Level	High Exposure Level
RH1, RH2	LEV	LEV and RPE	LEV and RPE or specially designed hood with LEV
RH3	LEV and RPE	LEV and RPE	Specially designed hood with LEV. Glove bag and RPE.
RH4	Glove bag and RPE	Designed hood with LEV, glove bag, and RPE	Isolation equipment. Down flow booths. Fume hood fitted with hand ports and gloves.
RH5	Isolation equipment. Down flow booths. Fume hood fitted with hand ports and gloves. Special decon.	Isolation equipment. Glove boxes	Isolation equipment. Glove boxes
RH6	Isolation equipment. Glove boxes	Isolation equipment. Glove boxes (<100g)	Isolation equipment. Glove boxes (<10g)

LEV = Local Exhaust Ventilation

RPE = Respiratory Protective Equipment

TABLE 5
E.U. RISK CLASSIFICATION PHRASE TABLE

<u>RH Hazard Category</u>	<u>European Union (EU) Risk Plan</u>	<u>E.U. Classification</u>	<u>Risk Phrase Number</u>
1	Not classified		
1	Harmful by inhalation (particulates)	X _n	R 20
1	Harmful if swallowed	X _n	R 22
2	Harmful by inhalation (vapors)	X _n	R 20
2	Toxic if swallowed	T	R 25
2	Toxic by inhalation/ if swallowed (particulates)	T	R 23
2	Irritating to respiratory system	X _i	R 37
3	Toxic by inhalation (vapors)	T	R 23
3	Very toxic by inhalation /if swallowed (particulates)	T+	R 26
3	Very toxic if swallowed	T+	R 28
3	Danger of very serious irreversible effects	T	R 39
3	Danger of serious damage to health by long term exposure	X _n	R 48
3	Possible risk of impaired fertility	X _n	R 62
3	Possible risk of harm to the unborn child	X _n	R 63
4	May cause cancer by inhalation	T	R 49
4	Very toxic by inhalation (vapors)	T+	R 26
[5] 4*	Danger of very serious irreversible effects	T+	R 39
[5] 4*	Possible risk of irreversible effects	X _n	R 40
[5] 4*	May cause sensitization by inhalation	X _n	R 42
[5] 4*	May cause cancer	T	R45
4	May impair fertility	T	R60
4	Danger of serious damage to health by prolonged exposure	T	R 48
4	May cause harm to unborn child	T	R 61
4	May cause heritable genetic damage	T	R 46

* Where an RH [5] 4 Hazard Category is indicated in the risk phrase table above, it is necessary to look at the data to determine the appropriate category.