Division of Public Safety

ENVIRONMENTAL HEALTH AND SAFETY

BIOSAFETY MANUAL

Revised January 2025

UNIVERSITY EMERGENCY CONTACTS

BIOSAFETY OFFICERS	**8 am - 5 pm**	**After 5 pm, Weekends**
Charlotte Waggoner	**540-231-5864**	**911***
Michael Miles	**540-231-3361**	
Anna Kroner	**540-231-1122**	
Allison Price	**540-231-8223**	
Sara Cilino	**540-231-8214**	

EMERGENCY TELEPHONE NUMBERS	**8 am - 5 pm**	**After 5 pm, Weekends**
Virginia Tech EHS Main Office **National Children's Hospital EHS**	**540-231-3600** **202-545-2702**	**911***
Hazardous Material Safety **Virginia Tech** **National Children's Hospital**	**540-231-2982/8758, 540-320-4754** **202-545-2702**	
Radiation Safety	**540-231-5364, 540-320-8305**	
Police/Rescue	**911***	
Fire Department		

*** If using a cell phone to dial 911, remember to identify your location for the operator.**

- **OFF-CAMPUS SITES SHOULD USE 911 FOR LOCAL EMERGENY RESPONSE.**
- **Use the non-emergency Virginia Tech Police number, (540) 382-4343, for information and to contact Virginia Tech support personnel as needed.**
- **Use the non-emergency National's Children's Hospital Police number, (202) 715-7400, for information and to contact support personnel as needed.**

INCIDENT / ACCIDENT REPORTING

Copy this section and place it near the lab's telephone.

1. As soon as any initial response is complete and the incident is stable, ***immediately notify:***
    a. The Lab Director and/or Lab Manager
    b. The Animal Facility Manager/ Greenhouse Manager (if applicable)
    c. A Biosafety Officer (BSO) by telephone (preferred) or email.

2. The BSO will acknowledge receipt of notification by communicating to the reporting person via phone or email, and will begin notifying other appropriate personnel and/or agencies.

3. **IMPORTANT:** If the incident involves a known exposure to recombinant material (e.g., rDNA/ synthetic nucleic acids/ transgenic or genetically modified organisms), the BSO must inform NIH without delay, thus IMMEDIATE reporting is required.

4. The reporting person and the supervisor of the facility (e.g., Lab Director/ Lab Manager/ Animal Facility Manage/ Greenhouse Manager) must complete a **VT Lab Incident Report** and submit it to the BSO via email (preferred) or campus mail (MS 0423) **as soon as possible**.

5. BSO will acknowledge receipt of this report via email.

6. **If an injury or exposure has occurred**, an **Employer's Accident Report** must be completed immediately by the supervisor per directions found on the link webpage.

7. If the supervisor does not complete the report in a timely manner, injured/exposed individuals are encouraged to complete the Employer's Accident Report themselves.

Biosafety Officers	**Telephone**	**Email**
Charlotte Waggoner	540-320-5864	ren@vt.edu
Michael Miles	217-377-4610	msmiles@vt.edu
Anna Kroner	540-525-8574	akroner@vt.edu

Table of Contents

ACRONYMS

ABO -- Associate Biosafety Officer

ARO -- Alternate Responsible Official

BBP -- Bloodborne Pathogens

BI -- Biological Indicator (autoclave verification testing)

BMBL -- *Biosafety in Microbiological and Biomedical Laboratori*es, 5th Ed.

BSC -- Biosafety Cabinet

BSL -- Biosafety Level

CDC -- Centers for Disease Control and Prevention

CI -- Chemical Integrator (autoclave verification testing)

EAR -- Employer's Accident Report

ECP -- Exposure Control Plan

EHS -- Environmental Health and Safety

IACUC -- Institutional Animal Care and Use Committee

IBC -- Institutional Biosafety Committee

IRB -- Institutional Review Board

LSBM -- Lab-Specific Biosafety Manual

NHP -- Non-Human Primate

NIH -- National Institutes of Health

OSHA -- Occupational Safety and Health Administration

PI -- Principal Investigator

PPE -- Personal Protective Equipment

RG -- Risk Group

RMW -- Regulated Medical Waste

RO -- Responsible Official

SDS -- Safety Data Sheet

UBM -- University Biosafety Manual

UBO -- University Biosafety Officer

DEFINITIONS

Aseptic Technique – (sometimes referred to as **good microbiological technique**) -- Work methods designed to reduce or prevent contamination by microorganisms in the environment when working with cultures of microorganisms, sterile materials or sterile areas; elements of aseptic technique are 1) use and maintenance of sterile work areas and containment equipment (BSC), 2) good personal hygiene, 3) use of sterile reagents and media, and 4) sterile handling methods for equipment, instruments, supplies and other materials.

Biohazardous Materials – A biological agent or condition that constitutes a hazard to human beings or the environment, including:

- infectious agents (bacterial, viruses, protozoans, fungi, etc.),
- biotoxins
- human or non-human primate blood, fluids, cells or tissue cultur,
- recombinant DNA and synthetic nucleic acid molecules
- transgenic microbes, animals, invertebrates and/or plants
- select agents
- prions
- opportunistic pathogens
- poorly characterized or uncharacterized cell lines, tumors or other tissues
- cell lines and research materials from other labs that have not been verified
- replication defective pathogen vectors
- attenuated strains and fixed materials that have not been tested to verify the attenuation or inactivation of the material

Biohazard Risk Assessment – A summary of pathogenic and epidemiologic information for a biohazardous agent, compiled as a safety reference document for users of that agent. Biohazard risk assessments are among the required documents that comprise a *protocol* for a proposed bioresearch project; PIs must submit research protocols to the IBC for project approval. PIs are responsible for initially creating biohazard risk assessments, although they may be revised in the IBC project review process.

Biosafety – The application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to infectious/biohazardous agents and materials.

Biosafety Cabinet – (sometimes called **biological safety cabinet**) – An enclosed laboratory work surface equipped with filtered directional air flow. BSCs provide the best place to work with biohazardous materials because they protect workers and the environment from exposure to agents, and protect cultured agents and sterile items from airborne contaminants.

Biosafety Levels – Combinations of laboratory practices, safety equipment and facilities features that define the conditions under which infectious agents can be safely manipulated. Levels of containment range from the lowest Biosafety Level, BSL-1, to the highest at BSL-4, where the most aggressive measures are taken to prevent exposure and release of biohazardous agents. At Virginia Tech, most research laboratories operate at BSL-1 and BSL-2. Two BSL-3 facilities are maintained by highly trained laboratory personnel. Virginia Tech has no BSL-4 facility.

Summary of Recommended Biosafety Levels for Infectious Agents (from BMBL, 5th Ed.)

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	■ No primary barriers required. ■ PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required
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 manipulations of agents that cause splashes or aerosols of infectious materials ■ PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: ■ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease 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: Protective laboratory clothing, gloves, face, eye and 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 ■ Entry through airlock or anteroom ■ Hand washing sink near laboratory exit

Biosafety Officer/ Associate Biosafety Officer – Individuals responsible for establishing and monitoring workplace safety procedures designed to minimize or prevent exposures and releases of biohazards.

Biotoxin – (also known as **biological toxin**) – Toxic substances of biological origin that may cause death or severe incapacitation at relatively low exposure levels. To utilize or produce biotoxins in research, PIs must have IBC approval and adhere to explicit safety and 'Due Diligence' requirements. Certain biotoxins have been designated as select agents.

Decontamination – A process or treatment that removes or neutralizes biologic, chemical or radiologic contamination from a person, object or area. In the biosafety realm, this term can be correctly applied to the autoclaving of biological lab waste prior to disposal, or chemically treating a lab surface with a disinfectant. The term can refer to achieving an *acceptable reduction* of microbial contaminants, whereas sterilization refers to a *complete elimination* of microbes.

Employer's Accident Report Form – The Virginia Tech form that injured employees are expected to complete in order to report an accident or illness that has arisen out of (or during the course of) their employment; completed forms are submitted to VT Human Resources. This form must be completed as soon as possible for the injured employee to be eligible for workers' compensation.

Exposure – when a biohazardous material has made contact or may have made contact with:

- a puncture injury site on your body (e.g., needlestick or cut with sharp object)
- the mucous membranes of your eyes/ nasal passages/ mouth
- non-intact skin surface (e.g., exposed skin that is chapped, abraded, has dermatitis, etc.)

Institutional Biosafety Committee (IBC) – The oversight body tasked with ensuring that instructional and research activities involving biozardous materials at Virginia Tech are in compliance with federally-mandated responsibilities and obligations for proper containment and safe handling of biohazards.

Incident – An event that results in an undesired circumstance. For a lab conducting research using biohazardous materials, examples of incidents are:

- spills, in or out of containment
- potential or known exposures
- loss of containment
- release of biohazardous agent into the environment
- injuries/illness caused by lab activities
- breaches of biosecurity
- any combination of these

Laboratory Incident Report Form – The Virginia Tech form to be completed by Principal Investigators, Lab Managers and/or lab personnel after the occurrence of an incident/accident in the laboratory; completed forms are submitted to EHS.

Personal Protective Equipment – Protective clothing, headgear, eyewear or other garments or equipment designed to protect the wearer's body from physical injury or exposure to hazardous substances/agents. Hazards addressed by PPE include physical, electrical, temperature extremes, chemical, biohazards, and airborne particulates. Typical PPE for biosafety in laboratory research: disposable gloves, lab coat or disposable gown, safety glasses/goggles, face shield, insulated gloves for autoclaving or working with liquid nitrogen storage, respirator for working with respiratory pathogens.

Principal Investigator – The primary individual responsible for the preparation, conduct and administration of a research grant, for achieving technical success of the research project, and for oversight of laboratory operations and personnel involved in working on the research project.

Primary Barrier – Safety devices which are physical barriers which are used to prevent direct contact between a hazardous agent/material and the worker. These include biosafety cabinets, personal protective equipment, etc. which are intended to protect laboratory workers.

Responsible Official (RO)/ Alternate Responsible Official (ARO) – The individuals who are accountable for Virginia Tech's Select Agent Program and its compliance with federal select agent regulations. The RO must be familiar with the regulations, have authority to act on behalf of Virginia Tech regarding the Select Agent Program, maintain required records, and conduct annual inspections. The ARO serves as RO when the RO is not available.

Risk Assessment – The process of researching, evaluating and identifying hazards/potential hazards in procedures, experiments, materials handling, etc. Risk must be assessed in every step of a procedure. Risk assessments also must determine the actions and controls required to eliminate or reduce risks to workers.

Secondary Barrier – Consists of a variety of safety features and devices (air filtration systems, decontamination equipment, facilities design features, etc.) meant to prevent exposure to/release of hazardous agents/materials if primary barriers fail.

Select Agents – (also known as **Select Agents and Toxins**) – Bioagents that have been declared by the U.S. Department of Health and Human Services or by the U.S. Department of Agriculture to have the "potential to pose a severe threat to public health and safety." At Virginia Tech, select agents can only be utilized in research with IBC approval and within the parameters of the Select Agent and Toxins Program.

Sharps – An object that can pierce or cut skin, causing injury and possible hazardous exposure. Higher risk sharps include needles, scalpel blades, razor blades, glass Pastuer pipettes, broken glass, glass slides, etc.;

lower risk sharps capable of piercing non-rigid containment and creating an exposure risk or minor injury include plastic pipettes, tips, wood applicator sticks, etc.

Spill Kit (Biological) – Materials assembled in a leakproof bucket or lidded box for use in cleaning up a spill of a biohazardous material in the laboratory. The Spill Kit should be well-labeled, and a wall sign should indicate the location of the Biological Spill Kit in the lab, particularly if it is kept under the bench, so it can be located quickly. Contents of the Biological Spill Kit: Disposable PPE (lab coat, gown or apron, gloves, shoe covers, face shield), absorbent paper towels, dustpan, tongs/forceps, autoclave bags, disinfectant, copy of spill cleanup procedure, spill warning sign to post. Respiratory protection should be provided in labs where agents are used that would present a respiratory hazard when spilled.

Standard Microbiological Practices – Basic hygiene practices or guidelines for working safely with microorganisms, including:

- Wash hands after working with potentially hazardous materials and before leaving lab.
- Do not consume food/drink, smoking, applying cosmetics or contact lenses, and no storing food in the lab.
- Do not mouth-pipette.
- Safely handle and dispose of all sharps.
- Minimize splashes and aerosols.
- Decontaminate biological material before disposing of it.
- Post biohazard signage on lab entrance when working with infectious agents.
- Maintain an effective pest management program.
- Ensure that lab personnel receive appropriate training and information that may be relevant to their particular health status.

Sterilization – The complete elimination of microorganisms to achieve a sterile, microbe-free condition; this can be done using a physical procedure (e.g., autoclaving) or chemical procedure (e.g., treatment by a strong disinfectant).

TERMS RELATED TO BLOODBORNE PATHOGENS

AIDS -- Acquired Immune Deficiency Syndrome, the disease that results when the HIV virus attacks the human immune system.

Blood -- Human or non-human primate blood, blood components, and products made from blood.

Bloodborne Pathogens -- Pathogenic microorganisms that can be present in human blood and can cause disease in humans. These pathogens include, but are not limited to:

- Hepatitis B Virus (HBV)
- Human immunodeficiency Virus (HIV)
- Hepatitis C Virus (HCV)
- Human T-Lymphotrophic Virus Type 1
- *Plasmodium* species (microbial parasite) causing malaria
- *Treponema pallidum* (bacteria) causing syphilis
- *Babesia microti* (microbial parasite) causing babesiosis
- *Brucella* species (bacteria) causing brucellosis

- *Leptospira interrogans* (bacteria) causing leptospirosis
- arboviral infections
- *Borrellia* species (bacteria) causing relapsing fever
- Creutzfeldz-Jakob disease (prions)
- Viral hemorrhagic fever

Clinical Laboratory -- A workplace where diagnostic or other medically-oriented screening procedures are performed on human blood or other potentially infectious materials, and differs from a research laboratory, where scientific investigations are conducted which can involve a wide variety of hazardous energy sources and materials, including biohazards.

Contaminated Sharps -- Any blood-contaminated object that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, and exposed ends of dental wires.

Engineering Controls -- Equipment or devices that isolate or remove the bloodborne pathogens hazard from the workplace. Examples include: sharps disposal containers, self-sheathing needles, equipment splash guards, biosafety cabinets, etc.

HBV -- Hepatitis B Virus, a bloodborne pathogen that may cause inflammation of the liver; acute infection can last < 6 mo. with complete recovery; chronic infection lasts > 6 mo. and can cause cirrhosis, liver cancer and death.

HCV -- Hepatitis C Virus, a bloodborne pathogen that causes serious liver damage and can be fatal; initial infection can occur with mild or no symptoms; chronic infection results in active liver disease, cirrhosis, liver cancer.

HIV -- Human Immunodeficiency Virus, the bloodborne pathogen that attacks the immune system and ultimately causes AIDS.

Non-Human Primate -- All non-human members of the order Primates, including, but not limited to animals commonly known as monkeys, chimpanzees, orangutans, gorillas, gibbons, apes, baboons, marmosets, tamarins, lemurs and lorises.

Occupational Exposure -- Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials which may result from the performance of an employee's duties.

Other Potentially Infectious Materials (OPIM)

The following human body fluids:

- semen
- vaginal secretions
- cerebrospinal fluid
- synovial fluid
- pleural fluid
- pericardial fluid
- peritoneal fluid
- amniotic fluid

- saliva in dental procedures
- any fluid mixed with blood
- any unknown body fluid

The following human tissues:

- any unfixed tissue
- any unfixed organ (other than intact skin)

The following research media or materials:

- HIV-containing cell culture
- HIV-containing tissue/organ culture
- HIV- & HBV- containing culture media
- human cell lines
- infected research animal tissue

Regulated Medical Waste -- A waste stream regulated by the Department of Environmental Quality which must be disposed of through EHS, even if it has been autoclaved or treated with another form of decontamination. Regulated Medical Waste includes:

- **Cultures and stock of microorganisms and biologicals** -- Discarded cultures, stocks, specimens, vaccines and associated items *likely* to contain organisms *likely* to be pathogenic to healthy humans.

- **Blood and blood products** -- Wastes consisting of human blood, human blood products and items contaminated by human blood.

- **Human tissues and other anatomical wastes** -- All human anatomical wastes and all wastes that are human tissues, organs, body parts, or body fluids.

- **Sharps** -- It is university protocol to include all sharps in the regulated medical waste stream, including ALL hollow-bore needles, pipettes, and glassware from biological labs or medical settings.

- **Some animal carcasses, body parts, bedding, and related wastes** -- Animal carcasses, body parts, bedding, and related wastes *if* the animal has been intentionally infected with pathogenic organisms and are *likely* to be contaminated.

- **Regulated Medical Waste *EXEMPTIONS***
  The following waste streams are **not** subject to the requirements of regulated medical waste regulations when dispersed among other solid wastes and not accumulated separately:
  - **Used products for personal hygiene**, such as diapers, facial tissues and sanitary napkins.
  - **Material**, not including sharps, **containing small amounts of blood** or body fluids, but containing no free flowing or unabsorbed liquid(Band-Aids).

Universal Precautions -- A method of infection control—recommended by the CDC—in which all human blood, certain body fluids, as well as fresh tissues and cells of human origin are handled as if they are known to be infected with HIV, HBV, and/or other blood-borne pathogens.

Work Practice Controls -- Procedures that reduce the likelihood of exposure through the manner in which tasks are performed.

INTRODUCTION

THE MANUAL HAS A NEW FORMAT

- This revised **University Biosafety Manual** incorporates information applicable to both BSL-1 and BSL-2 labs, and is now a reference-only document, containing no fillable content areas.
- All fillable content areas for your lab-specific information are now located in a separate document, the **Lab-Specific Biosafety Manual** (LSBM) template.

What the new University Biosafety Manual (UBM) does for you:

- Supplies current, relevant biosafety information, policies and practices for using biohazardous materials in the laboratory; content will be updated on a continuing basis by the EHS Biosafety Office.
- Documents the practices to use that minimize or eliminate exposure risks.
- Serves as an on-line reference for bioresearch lab personnel at Virginia Tech; no need to print a paper copy unless you choose to do so.
- Provides essential material for training new personnel before they perform work in the lab.
- Provides links to all necessary forms and templates that are referenced within this document.
- Facilitates compliance of research using biohazardous materials with the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition and NIH Guidelines for Research Involving Recombinant DNA Molecules, as well as with the OSHA Bloodborne Pathogens Standard 1910.1030 for those laboratories utilizing human cell lines, human blood/ tissue specimens or other potentially infectious materials.

Completing The Lab-Specific Biosafety Manual (LSBM) Template:

- **Principal Investigators or their designees must complete the LSBM.**
- Read the online this document (UBM) to expedite LSBM completion.
- Electronically access the **Lab-Specific Biosafety Manual** template and provide information that is specific to your lab using fillable text areas and check boxes in Sections 1-19.

- Electronically send the document with completed Sections 1-19 to the IBC; this is a required component of your protocol submission. The IBC Administrative Office will populate LSBM Appendices A, B, C, D and E with other materials you have submitted in your protocol.

- Once it has been reviewed and approved, print a copy of your LSBM to keep in your lab.

- Update the LSBM when anything changes such as personnel, agents, procedures, equipment, work locations, etc. Document all updates on the *Manual Review/ Revision Status* page, and print the necessary updated pages to include in your printed LSBSM.

- The LSBM must be reviewed annually by PI or designee. Document your annual reviews on the *Manual Review/Revision Status* section.

REQUIRED PROCEDURES AND PRACTICES

1. INCIDENT/ ACCIDENT RESPONSE

1.1 First Response

- Give priority to addressing injury/medical emergencies, then address containment and cleanup. If the situation is life-threatening, treatment must be obtained in the Emergency Room of the nearest hospital; DIAL 911.
- Following an exposure to an infectious agent, elapsed time can be critical, so act quickly. In some instances, prophylactic medications can be given within the first few hours of exposure which will significantly lessen infection risk.
- **Immediately report any incident, accident or potential exposure to the PI, Lab Manager or other emergency contact for the laboratory. That person will advise and direct the appropriate course of action. TO REPORT A BIOHAZARDOUS EXPOSURE, INCIDENT OR ACCIDENT, REFER TO THE INCIDENT/ACCIDENT REPORTING PROCEDURE AT THE BEGINNING OF THIS MANUAL, page iii.**

1.2 Physical Injury

- Provide immediate first-aid: stop bleeding of wounds and, if appropriate, wash the affected area with disinfectant/soap.
- If the incident is a medical emergency, DIAL 911 immediately. **Tell the first responders if there is a potential for contamination, and the biohazardous materials involved.**
- **Decontaminate yourself and others as effectively as you can prior to seeking medical attention.**
- **Access the specific risk assessment sheets and/or SDS sheets if chemical or biohazardous agents are involved. Give this documentation to emergency response personnel caring for the victim; *emergency crews and hospital staff will need these to provide appropriate medical treatment.***
- **For any injury or exposure incident involving biohazardous materials that requires the non-emergency services of primary health care providers, BSL-2 risk assessments and/or SDS sheets must be provided so that appropriate treatment can be safely administered.**

1.3 Eye Exposure

- Immediately flush eyes for 5-10 minutes using an eyewash station. Seek medical attention after flushing.

1.4 Needle Stick or Open Wound Exposure

- Clean and wash area thoroughly with soap and water for a minimum of 5 minutes. If injury is a needlestick, gently massage the area to make it bleed while washing. If desired, follow with an application of alcohol-based gel hand sanitizer. Bandage the wound as needed.
- Go to an appropriate healthcare provider for evaluation (Emergency Room, primary care physician, Student Health Services, etc.). Inform the health care provider of the biohazardous material(s) to which you have been exposed and bring risk assessments/ SDSs.

1.5 Mucous Membrane Exposure (Eyes, Nose, Mouth)

- Immediately flush membranes if possible with plain water.
- Go to an appropriate healthcare provider for treatment (Emergency Room, primary care physician, Student Health Services, etc.) Inform the health care provider of the biohazardous material(s) to which you have been exposed and bring risk assessments/ SDSs.

1.6 Building Emergencies- Fire, Fire Drill Or Other Evacuation (Burst Pipe, Gas Leak, Etc.)

Note: Only follow the procedures below if it is safe to do so. Your building should have an Emergency Action Plan; see this document for further details. To access this document for more emergency response information, contact your building's emergency coordinator.

- Seal or close all open containers of infectious material and remove PPE.
- If working in a BSC or laminar flow hood:
    - Immediately suspend work. Seal or close all open containers of infectious material, remove PPE and close the sash if you can safely do so.
    - If there is time, attach a sign to the cabinet, "SASH MUST STAY CLOSED," until you can return to it.
    - Pull the nearest Fire Alarm and DIAL 911.
    - Evacuate the building.

1.7 Loss of Electrical Power When Working With Biohazards

- Any biological materials (e.g., samples, agents) in lab work areas other than in the BSC (e.g., centrifuge, water bath, sonicator, incubator) must be secured in containers or equipment, closed as well as possible, and posted with a DO NOT OPEN sign to prevent accidental exposure of emergency personnel or service personnel requiring access to the laboratory.

- If working in a BSC or laminar flow hood:

  1. Immediately stop work and seal any open containers of infectious material. Remain at the cabinet for a minute or two to see if power is restored. Avoid making fast arm movements in/out of the BSC to prevent pulling contaminants out of the cabinet.

  2. If power is not restored within a few minutes, remove PPE, close the cabinet sash and post a sign that identifies the biological materials being used therein and explicitly states that the sash should stay closed until power is restored.

  3. When power is restored, don appropriate clean protective clothing when approaching the BSC.

  4. Check to see if BSC function has been restored.

  5. Open sash and if necessary discard any items exposed to unfiltered air while power was off. Place items in biohazard bag and autoclave immediately.

1.8 Biohazardous Spills

1.8.1 Biological/ Biohazardous Spill Kits

- What supplies should be included in a Biological or Biohazard Spill Kit?

- Contents should be contained within a handled bucket and include a disposable lab coat or coveralls, disposable gloves, face shield/mask, protective footwear, spray disinfectant, clean-up supplies (forceps, dustpan, autoclave bags, spill pillows & socks) and a sign that reads" Biohazard Spill DO NOT ENTER".

- If a respiratory hazard is indicated through risk assessment of the BSL-2 agents used in the area, exposure protection in the form of respirators should be provided separately by the laboratory. N-95 respirators must be fit-tested by EHS. Contact EHS to arrange.

- The Spill Kit should be well-labeled, and a wall sign should indicate the location of the Biological Spill Kit in the lab, particularly if it is kept under the bench, so it can be located quickly.

- All personnel working with BSL-2 materials must receive training for Biohazard Spill Kit use.

1.8.2 MAJOR and Minor Spills

- **What is considered a MAJOR spill**?

  A MAJOR spill is one which, in your judgment, could represent a significant environmental risk or serious human health risk as a result of release or exposure, and/or is a larger-volume spill (> 2 L, for example) of biohazardous agents or recombinant/synthetic nucleic acids which is beyond the capacity and training of lab personnel to safely execute cleanup, and will require cleanup by haz-mat professionals.

- **What is considered a minor spill?**

  A spill of lesser volume (< 2 L, for example) and/or with agents of lesser pathogenicity, for which cleanup can usually be handled by lab personnel using absorbent materials/ disinfectants routinely kept on the bench or in lab spill kits.

- **How do I decide if a spill is major or minor?**

  Consider pathogenicity, concentration, aerosol hazard and/or environmental hazard of the material, and the volume of the spilled material when making this decision. Consult the risk assessment(s) for the agent(s) if necessary, but make the decision as quickly as possible.

- **WHAT TO DO IN THE EVENT OF A MAJOR SPILL:**

  1. Immediately notify everyone in the lab /area and clear the area of all personnel.
  2. Secure the area as needed by locking doors, standing guard to keep people out, posting signs, etc.
  3. Call 911 or 231-6411 for VT Campus Police; ask that EHS be informed immediately.
  4. Inform your PI or Lab Manager/Supervisor as soon as you possibly can.

  IMPORTANT:

  - **Chemical disinfectants require contact time with the spill to effectively decontaminate it. Be aware of the specific contact time of the disinfectant you use and allow that time to elapse before clean-up.**
  - For metal surfaces, follow all bleach disinfectant treatments with a water rinse.

- If a chemical disinfectant is not used (or cannot be used) with contaminated items, decontaminate by autoclaving or other method approved by EHS if items can withstand the process (e.g., contaminated lab coats).
- **For any spill involving broken glass**: DO NOT HANDLE BROKEN GLASS WITH YOUR HANDS. USE A DUSTPAN, FORCEPS OR OTHER DEVICE TO PLACE THE GALSS INTO AN APPROVED SHARPS CONTAINER TO BE AUTOCLAVED.

1.8.3 Spill Occurring Inside A Biological Safety Cabinet

1. Immediately notify everyone in the lab/area of the spill. Remove any contaminated PPE/clothing and place in Biohazard bag to be autoclaved.

2. Put on clean disposable PPE prior to initiating clean up.

3. Continue operating biosafety cabinet blower to help control any aerosols.

4. Lesser spills, even the smallest amount, should be **immediately** treated with the appropriate disinfectant for your lab. After sufficient contact time, wipe up with paper towels.

5. Surfaces treated with bleach should be rinsed immediately with sterile water to avoid damage to the surface metal of the cabinet.

6. Spills of greater volume require more extensive decontamination of cabinet surfaces with greater volumes of the appropriate disinfectant for your lab. Allow sufficient contact time, and clean up with absorbent materials followed by a sterile water rinse. Use Spill Kit if necessary.

7. Inform all users of the BSC, as well as the Laboratory Manager and/or PI, about the spill and status of clean-up as soon as possible.

8. For a major spill of BSL-2 material within a cabinet, the cabinet's fan, filters and airflow plenums may need to be decontaminated by formaldehyde gas procedures. Contact the biosafety cabinet service contractor to schedule this procedure.

9. *DISPOSAL OF LARGE AMOUNTS OF ABSORBENT AND CLEANING MATERIALS SATURATED WITH DISINFECTANT (FROM A LARGE SPILL CLEAN UP):*
    a. Collect in a blue Chemical Waste bag; contact EHS for disposal.
    b. Avoid overfilling bags; bags should be closed securely.
    c. Place bags in secondary, autoclavable containers (i.e., a Nalgene or stainless steel pan) until pickup.
    d. Spray bag surface liberally with 70% ethanol and allow to dry.

1.8.4 Spill Occuring Inside Lab And Outside Biological Safety Cabinet

- NOTIFICATION: Immediately notify everyone in the lab/area of the spill. Remove contaminated PPE/clothing and place in biohazard bag to be autoclaved.

- *MAJOR SPILLS:* ***Follow the procedure listed in 1.8.2.***

- *MINOR SPILLS:* Put on clean disposable PPE prior to initiating clean up. Clean up immediately with paper towels soaked in disinfectant, allowing for appropriate contact time.

1. Clear area of all personnel, apply absorbent material on spill if safe to do so, exit room, close door and mark it with 'BIOHAZARD SPILL/ DO NOT ENTER" (or post sign included in Spill Kit). Secure area as needed, including locking doors, standing guard to keep people out, etc.
2. Notify the PI or Lab Manager of the spill.
3. Wait 30 minutes for aerosols to settle before entering spill area. Assemble clean up materials from bench or use items supplied in spill kit (whichever is most readily available) and don PPE during this time.
4. Initiate clean-up as soon as possible following the 30 minute wait by placing absorbent material on spill and soaking spill area with the appropriate disinfectant for your lab. Work from the outside of the spill and finishing in the center.
5. Allow appropriate contact time (at least 20 minutes).
6. Pick up absorbent material and place it in a biohazard bag.
7. Repeat placing absorbent material and flooding with disinfectant until you are convinced the decontamination is complete (at least twice). Finish with a water rinse.
8. Place contaminated **reusable** items in biohazard bags, or lidded, heat-resistant pans/containers with lids before autoclaving. Place large equipment in separate bags and place bags on a lab cart for transport to autoclave. Initiate further clean-up, if needed, after autoclaving.
9. Expose non-autoclavable materials to disinfectant for 20 minutes.
10. Inform all lab personnel as well as the laboratory supervisor/principal investigator about the status of clean-up as soon as possible.
11. *DISPOSAL OF LARGE AMOUNTS OF ABSORBENT AND CLEANING MATERIALS SATURATED WITH DISINFECTANT (FROM A LARGE SPILL CLEAN UP)*
    a. Collect in a blue Chemical Waste bag; contact EHS for disposal.
    b. Bags should not be overfilled, and should be closed securely.
    c. Place bags in secondary, leakproof pan or container until pickup.
    d. Spray bag surface liberally with 70% ethanol or other appropriate disinfectant, and allow to dry.
12. *AUTOCLAVING CONTAMINATED, HEAT-RESISTENT ITEMS NOT TREATED WITH DISINFECTANT*
    a. Collect in a Biohazard autoclave bag.

b. Bags should not be filled more than 2/3 full. When full, close the bag securely.
c. Place bags in secondary, autoclavable containers (a Nalgene or stainless steel pan).
d. Spray bag surface liberally with 70% ethanol and allow to dry.
e. Immediately transport to autoclave/glassware room and decontaminate by autoclaving as soon as possible.
f. Immediately prior to autoclaving, loosen bag closure to allow steam penetration within bag.
g. Dispose of all decontaminated waste in Regulated Medical Waste boxes.

1.8.5 Spill Occurring In Work Area Outside Of Laboratory

1. Warn personnel in the immediate area of the spill. Block off spill area as best you can.
2. See section "Spill Occurring Inside Lab and Outside Biological Safety Cabinet" for instructions.

1.8.6 Spill Occurring Inside A Shaking Incubator

IMPORTANT:

- Immediately turn off power to unit and unplug power cord from wall socket.
- Immediately notify everyone in the lab/area of the spill.
- If spill volume is large (>2 L), then close lid of incubator and call PI or Lab <Manager for assistance.
- If spill can be safely contained and removed by lab personnel, proceed as follows:

1. Remove any clothing contaminated with spill and place in Biohazard bag to be autoclaved. If skin is contaminated, treat with non-bleach disinfectant and follow with a soap and water rinse.
2. Quickly place paper towels on spill inside incubator to absorb liquid before it leaks onto motorized parts below, then close lid.
3. Ask someone to contact the PI or Lab Manager to advise them of the spill while you retrieve: a) the Biohazard Spill Kit from its storage location in the lab, and b) a sufficient quantity of appropriate disinfectant.
4. DO NOT LEAVE THE ROOM WITHOUT PUTTING A SIGN ON THE INCUABATOR THAT SAYS "BIOHAZARDOUS SPILL INSIDE—DO NOT OPEN OR USE!"
5. DO NOT MIX DISINFECTANT TREATMENTS IN YOUR CLEAN-UP, ESPECIALLY BLEACH AND ETHANOL.
6. If you are wearing no PPE, put on the PPE in the Spill Kit.
7. Check to see if liquid is leaking out from the unit onto bench or floor. If so, apply absorbent pads or paper towels soaked with disinfectant to the liquid, as well as in a perimeter around the spill; wait for appropriate contact time, then clean up with paper towels or other absorbent material from Spill Kit. Discard soaked material into Biohazard waste.

8. Remove the pieces of broken vessels from the incubator interior; use forceps to avoid skin injury. Place broken glass in Sharps container; decontaminate by autoclaving as soon as possible.

9. Now direct your attention to the interior of the incubator once again. Spilled liquid cannot be absorbed all at once because of the way shaking incubators are constructed, therefore you must work from top to bottom. Spray disinfectant over the soaked paper towels you applied earlier, then position a Biohazard autoclave bag as close as possible for discarding the towels. Place wet towels in bag carefully, minimizing aerosols and drips however possible.

10. Immediately apply more absorbent material to the spill if needed. Use pads, socks or pillows from the Spill Kit according to the volume of the spill and the size of the area to cover.

11. Spray interior surface areas of unit with disinfectant, especially any pieces of broken vessels associated with the spill. Wait for disinfectant to be effective.

12. At this point you may need to remove the incubator's platform to get to lower regions for further spill cleanup. Removal is often accomplished by using a hexagonal T wrench on 4 platform screws; a Phillips screwdriver may be needed to move flask clamps if they are covering the platform's hex screws. These tools should be located on or near the shaking incubator.

13. Thoroughly spray platform with disinfectant before removal, and give disinfectant time to work.

14. Before taking platform out of incubator, spray paper towels with disinfectant and use them to cover an area on lab floor upon which to place the platform. Choose an area of the floor that is out of your way. Place removed platform onto paper towels and perform a more thorough clean up later. Spray tools with disinfectant.

15. Apply absorbent material to any spill liquid you see in lower regions of the incubator. TRY TO ABSORB AS MUCH OF THE SPILL FROM AS MANY SURFACES AS YOU CAN.

16. Flood the absorbent material in the incubator with enough liquid disinfectant to decontaminate, but not so much as will create a gross excess in the spill area. Wait for disinfectant to be effective.

17. Place absorbent material saturated with disinfectant into blue Chemical Waste bags and securely close the bags. Bags must be kept in secondary containers while awaiting pickup.

18. For materials that can be decontaminated by autoclaving (i.e., containing no alcohol or bleach), place directly into Biohazard autoclave bags and close bags securely. Place bags in secondary containers when transporting to autoclave room. Decontaminate by autoclaving.

19. After all spilled material has been removed, disinfect every surface of the incubator that is accessible and repeat if necessary. Use cotton-tipped swabs for hard-to-reach areas. Do not use bleach on metal parts.

20. Put cleaned, dried platform back into position.

21. Leave lid of incubator open for additional drying out.
22. Mop lab floor with disinfecting agent.
23. Autoclave any contaminated PPE.
24. Have a technical service provider test the incubator for electrical safety and proper function before returning it to service.

1.8.7 Spill Occurring Inside Centrifuge

1. Immediately notify everyone in the lab/area of the spill.
2. Leave centrifuge closed for at least 30 minutes for aerosols to settle. During this time, get clean-up supplies ready, including Spill Kit if needed.
3. Absorb spill with paper towels.
4. Soak spill area with the appropriate disinfectant for your lab. Allow contact time of 20 minutes. All exposed surfaces should be disinfected, including heads, cups, cushions, etc.
5. Saturate area again with disinfectant. Allow 20 minutes contact time, then repeat clean up and finish with a water rinse.
6. All disposable materials used in clean-up must be collected in a blue Chemical Waste bag. Place bag in secondary container and contact EHS for disposal.
7. Report the spill to your PI, Lab Manager, or designee.

1.8.8 Spill Occurring In Water Bath Or Shaker Bath

1. Immediately notify everyone in the lab/area of the spill.
2. Turn power off.
3. Pour the appropriate disinfectant for the organism directly into water bath in sufficient quantity to effect decontamination. (CAVICIDE is recommended over bleach to reduce likelihood of damage to metal parts from chloride exposure.)
4. Replace cover and wait for 20 minutes.
5. Discard the water/disinfectant solution by pouring down sink drain, and flush sink drain with water.
6. Disinfect the surfaces of the water/shaker bath, and allow to dry before returning unit to regular use.
7. Report the spill to your PI, Lab Manager, or designee.

1.8.9 Spill Occurring In Incubators Or Refrigerators

1. Minor spills which have not generated significant aerosols may be cleaned up with a paper towel soaked in disinfectant.
2. In the event of a major spill, the door should be left shut for 30 minutes to allow any aerosol to settle.
3. Absorb the spill with paper towels, and flood the area with the appropriate disinfectant for your lab. Allowing for a contact time of 20 minutes, all exposed surfaces should be disinfected, including equipment, racks, tubes, bottles, etc.
4. Repeat step 3 if needed. Finish clean up with a water rinse.
5. All disposables used in the clean up procedure should be collected in a blue Chemical Waste bag. Place bag in secondary container and contact EHS for disposal.
6. Report the spill to your PI, Lab Manager, or designee.

2. ROLES AND RESPONSIBILITIES

2.1 Principal Investigator

- **Research Compliance**
  - IBC Requirements apply to research with biohazardous materials; these requirements include:
    - Submitting initial research protocol applications for IBC review and approval, including Risk Assessments for biohazardous agents/materials, and pertinent Standard Operating Procedures involving direct manipulation of biohazardous agents/materials.
    - Submitting amendments to approved protocols as changes occur over the course of a research project
    - Performing a self-inspection of your laboratory as part of your IBC annual review
    - Submitting protocol renewal applications every three years
  - If animals are involved in your research, compliance with IACUC regulations will be required.
  - If human subjects are involved in your research, compliance with IRB regulations will be required.
  - Laboratory research compliance also is required with university, state and federal policies regarding health and safety, and environmental quality.

- **Laboratory Safety Practices, Supplies, Equipment**
  - Provide and maintain engineering controls, safety devices, etc. (e.g., handwashing facilities, needle safety devices, sharps containers, aerosol control, biosafety cabinets, PPE).

- Provide/ see to the maintenance of lab equipment.
- Be receptive to workers' suggestions for safety devices/products or improved safety procedures.
- Require that lab workers review with the PI any novel, untried, potentially hazardous procedures or innovations before trying them.

- **Personnel Supervision and Training**
  - Take all safety training required of those who work in the PI's laboratory.
  - If the PI so chooses, select a Laboratory Manager/Supervisor who can serve as the PI's designee to be responsible for daily oversight of the lab and for specific areas of responsibility as agreed upon by PI and designee.
  - Directly provide/ensure the provision of lab-specific safety training, procedural training and awareness training for personnel.
  - Ensure that personnel have completed required training and stay current with their training.
  - Assess and endeavor to improve lab worker proficiency.
  - Enable the receipt of any required in-class training, Hepatitis B vaccinations, and other immunizations or occupational health services for lab workers *during regular work hours*.

- **Documentation**
  - Ensure that lab workers complete and update medical surveys, when applicable.
  - Ensure that the LSBM for the lab is complete, current and accessible to lab workers.
  - Conduct an annual review of the LSBM, and reviewing it with lab workers annually and/or whenever changes are made.
  - Promptly report incidents, accidents, exposures and spills to EHS, and Human Resources if needed; document incidents, and maintain required records of incidents.

- **Lab Access and Safety for Non-Lab Workers**
  - Coordinate with Housekeeping supervisor to determine housekeeper access and duties.
  - Determine visitor and service provider access to the lab, and escort practices for them while they are in the lab.
  - Ensure that visitors, housekeepers, and service providers receive pertinent information on lab hazards and safety practices according to the procedures documented in this manual.

2.2 Lab Managers And All Who Work In The Lab

- Participate in all required training, and follow practices learned in training.
- Adhere to Universal Precautions.
- Read and understand the online UBM and your lab's LSBM, and know the location of the LSBM in your lab or work area.
- Follow all departmental and lab-specific safe work practices.

- Review any novel, untried, potentially hazardous procedure or innovation with the PI before trying it.
- Maintain clean and orderly work areas; clean up after yourself.
- Maintain laboratory equipment according to routine practices that ensure safe operation.
- Inform supervisors of problems with facilities, equipment, procedures, or coworkers.
- Conduct interactions with coworkers in ways that promote lab safety and good work practices.
- Report accidents, incidents, problems and near-misses to supervisors.
- Perform decontamination and containment practices including, but not limited to:
    - Routine decontamination of work surfaces and equipment
    - Decontamination and clean-up of spills
    - Decontamination and/or disposal of contaminated PPE
    - Decontamination of lab equipment scheduled for repair or surplus
    - Appropriate handling of lab hazardous waste
- Take personal ownership for all aspects of safety in your work.
- Follow security procedures for the laboratory, as designated by the PI.

2.3 Environmental Health and Safety

- Provide biosafety-related information, guidance, consultation and assistance to the Virginia Tech research community.
- Facilitate bioresearch compliance with federal, state and local regulations.
- Perform biosafety lab inspections and general safety inspections for campus buildings.
- Serve as liaison and advocate for research labs with regulatory agencies.
- Provide biosafety-related training.
- Package and ship all biohazardous materials being sent from Virginia Tech to national/ international destinations.
- Collaborate with others at Virginia Tech to promote an integrated safety culture and safe working environment.

2.4 Institutional Biosafety Committee

- Conduct reviews for compliance with the National Institutes of Health Guidelines, and grant approval for research and teaching proposals using the following materials:
    - Recombinant DNA and synthetic nucleic acids
    - Transgenic organisms
    - Artificial gene transfer
    - Infectious microbial agents
    - Human, NHP and mammalian blood, blood products, cells and unfixed tissue
    - Toxins of biological origin

  - Select agents and toxins
  - Prions
  - Dual-use technologies
  - Synthetic biology
- Approve, disapprove or require modifications in these research protocols to ensure regulatory compliance with federal and state regulations.
- Suspend or terminate ongoing protocols in which unacceptably hazardous activities involving biohazardous materials are taking place.
- Provide regulatory/ biosafety oversight for activities conducted by Virginia Tech research/teaching endeavors using these materials.

2.5 Institutional Animal Care and Use Committee

- Review proposed uses of vertebrate animals in research, testing or education at Virginia Tech.
- Approve, disapprove or require modifications in these research protocols to ensure regulatory compliance with federal and state regulations.
- Suspend or terminate ongoing protocols in which inappropriate or unapproved activities involving animals are taking place.
- Provide regulatory oversight for activities conducted in Virginia Tech research and teaching which utilizes vertebrate animals.

2.6 Institutional Review Board

- Review research protocols which involve human subjects to ensure their rights, safety and privacy.
- Approve, disapprove or require modifications in these research protocols to ensure regulatory compliance with federal and state regulations.
- Suspend or terminate ongoing protocols in which inappropriate or unapproved activities involving human subjects are taking place.
- Provide regulatory oversight for activities conducted in Virginia Tech research and teaching which involve human subjects.

3. FACILITY SAFETY REQUIREMENTS

3.1 Signage

Biohazard Signs or Labels must be posted in/on:

- entrance doors to biological laboratories/ work areas (BSL-1, BSL-2)
- biosafety cabinets
- equipment (centrifuges, refrigerators, freezers, etc.) used with biohazardous materials
- transport containers for biohazardous materials

- secondary containers for biohazardous waste
- any other equipment used to store or manipulate biohazardous materials, and sample containers for biohazardous materials

3.2 Hand Washing Station

Laboratories must have a sink for hand washing. The sink may be manually, hands-free or automatically operated. It should be located near the exit door.

3.3 Biological Spill Kit

- Laboratories and related work areas handling biohazardous materials must have access to a Spill Kit which meets the specific needs of a biohazardous spill.
- Contents should be contained within a handled bucket and include:
    - disposable lab coat or coveralls
    - disposable gloves
    - face shield/mask
    - protective footwear
    - spray disinfectant
    - clean-up supplies (e.g., forceps, dustpan, autoclave bags, spill pillows & socks)
    - sign that reads "Biohazard Spill DO NOT ENTER"
- Expiration date on disinfectant must be checked periodically; expired disinfectant must be replaced.
- If respiratory hazard is indicated on Risk Assessment Forms for the BSL-2 agents in question, respiratory protection in the form of respirators must be provided separately by the laboratory; it will not be included in the Spill Kit. These units must be fit-tested by EHS. Contact EHS to arrange.
- All personnel working with BSL-2 materials must receive lab-specific training for Biohazard Spill Kit use.

3.4 Required Maintenance Of Safety Equipment In Facility

3.4.1 Eyewash Station

- Eyewash must be flushed for a minimum of 5 minutes weekly. For correction of functional problems or for repairs, contact University Facilities or your building's Facilities manager.
- A log sheet documenting weekly flushes per eyewash must be kept by the Laboratory Manager or other designated personnel.

3.4.2 Safety Shower

- EHS verifies proper functioning of safety showers on an annual basis. For correction of functional problems or for repairs, contact University Facilities or your building's Facilities Manager.

3.4.3 Fire Extinguishers

- Extinguishers which are located in laboratories *must be checked monthly for proper charge by lab personnel*.
- Those located in hallways are checked monthly by custodial staff, building maintenance/ Facilities staff, or other designated personnel.

3.5 Laboratory Security and Personal Responsibility

- The access doors to BSL-2 work areas must be closed when any work is being performed with BSL-2 materials.
- All access doors to the BSL-2 area must be locked when no one is in the lab for an extended period of time.
- Storage of BSL- 2 material that is located in an area accessible to the public or to any non-BSL2 lab personnel must be kept locked unless removing or adding material.
- If your physical/mental condition at any given time is compromised in some way (e.g., sleep-deprived, ill, exhausted, emotionally distraught, etc.) such that you are more likely to make serious mistakes, then postpone and reschedule any complex manipulations with biohazardous materials that you may have planned to do in the lab.
- If you need to work in the lab outside of regular hours, such as at night, or on weekends/holidays, either arrange to work with a 'buddy,' or let a responsible person know what your timeframe for working in the lab will be. Pre-arrange with that person to 1) check on your status if you have not left the lab or arrived at your home by your stated time, and 2) contact your supervisor if it seems your safety could be in question.

3.6 Restricted Entry For Health Reasons

The PI must discuss lab hazards with individuals who wish to enter the lab area but have health concerns regarding immunocompetency. If circumstances warrant, and/or in consultation with the Occupational Health physician or nurse, the PI can restrict access to the lab for such individuals for the sake of their health and well-being.

3.7 Visitor Access To / Presence In The Lab

- Visitor access will be determined by the PI.
- Prior to entry into the laboratory, all visitors must be informed of:
    - Basic emergency evacuation procedures.
    - Health hazards specific to the work occurring in the lab, as well as specific safety practices for avoiding those hazards. They must be told to avoid physical contact with all research equipment, material and working surfaces, unless invited and/or approved to do otherwise by authorized personnel, who will provide appropriate supervision.
- Infrequent visitors (i.e., those who enter the area less than once/month) must be made aware of the above items upon each visit.
- Frequent visitors (i.e., those who enter the area at least once/ month) can be informed initially, and updated as needed if conditions change in the lab.
    - If visitors' time spent in the lab will exceed a short stay, they will be:
        - Shown the emergency exit route from laboratory
        - Given a review of the Emergency Procedures list posted in the lab
        - Shown the locations and proper use of emergency eyewash & shower
        - Shown the locations of the nearest fire alarm and extinguisher
- To minimize possible liability issues, it is highly recommended that documentation be kept which would record dates/times of these visits, and verify visitors' receipt of lab hazard and emergency response information (i.e., a visitor sign-in/ sign-out/ receipt-of-training log).

3.8 Housekeeping And Service Personnel Access To/ Presence In Lab

- Presence of housekeepers in the laboratory should be kept to a minimum.
- PIs must coordinate with housekeeping supervisors to schedule housekeeping services that will be provided for the lab, and the frequency of service. Custodians/ housekeepers can be responsible for:
    - Emptying regular household trash
    - Regular sweeping and mopping of floors
- PIs or their designees are responsible for informing housekeeping supervisors of any safety awareness issues for housekeepers entering their labs and performing their duties.

- When repairs or other work needs to be done in the lab by service providers/facilities/movers:
    - Lab hazards must be secured in the service providers' work area.
    - The area should be cleared, cleaned and decontaminated (if applicable) by the date that the work is to be done.
    - The PI or designee will either:
        - Post a '*Clearance for Lab Access'* form on main lab entry, indicating that preparations have been made for workers' safety, and documenting any hazards present that they need to be informed of. (*Clearance for Lab Access* form can be found in the APPENDICES of this manual, and on the EHS website.)
        - Ensure that a responsible person from the lab will serve as escort for service worker entry into the lab, and convey pertinent hazard information to workers.
- To minimize possible liability issues, it is highly recommended that documentation be kept which would record dates/times of receipt, and a brief description of any lab-specific hazard awareness training provided to custodial and service personnel.

3.9 BSL-1 And BSL-2 Activities Within The Same Lab Work Space

- When separate BSL-1 and BSL-2 work is performed in the same laboratory space, the PI or designee will be responsible for ensuring that all lab workers in that space are aware of the hazards associated with the BSL-2 agents in use, and know how to avoid exposure.

3.10 Working With Different Biological Agents In Shared Lab Spaces

- As more bioresearch groups work in open or shared lab spaces and use common equipment, there is an increasing need for sharing biohazard information across the groups.
- Be aware that biosafety measures specific to your lab group are based in part on the risk assessments for the agents used, and are not necessarily applicable to other lab groups using biological agents with whom you share physical lab space; they will have their own lab-specific biosafety measures.
- Lab personnel must receive awareness training on the agents/materials used by others with whom they share work spaces, and others must receive awareness training on the agents/materials used by your lab group. PIs or their designees will provide this training. See Section 5 for details.

3.11 Maintaining The General Condition Of The Lab Facility

- Maintaining tidy, clean floors, sinks, benches and equipment, and well-organized/ inventoried shelves, cabinets and supplies is essential for successful biological research, and primarily will be the responsibility of laboratory personnel.

- Specific cleaning practices will be determined by PI and Lab Manager, and must be followed by all lab personnel.

- Problems observed by lab personnel involving the lab air handling, plumbing, electrical power, security or lighting systems must be immediately referred to the PI, Lab Manager or designated person. PIs, Lab Managers or designees will communicate problems to, and request repairs from the appropriate university Facilities service group for that location.

3.12 Integrated Pest Management

- Insect and rodent pests present a contamination risk and containment breech in laboratory areas, therefore an integrated pest management program is an important part of managing a research facility.

- The most common approach to pest control is the application of pesticides as a preventive or remedial measure. This can be effective as a corrective action, but pesticide use has limited long-term effects when used alone. In addition, pesticides can contaminate the research environment via volatilization.

- To minimize the presence of pests and the use of pesticides in the lab, a comprehensive effort is required that integrates housekeeping, maintenance and pest control, and is the responsibility of the PI and laboratory personnel to manage this integration, as each situation necessitates. An integrated pest management program prevents pest problems by managing the facility environment to make it less conducive to pest infestation in the following ways:

    - Food and drink, and food/drink storage are not allowed in any BSL-1 or BSL-2 space.
    - Routine cleaning and mopping of floors must occur.
    - The lab may place insect bait traps as needed, but must regularly monitor and replace them.
    - Lab workers must maintain a daily visual awareness for the presence of vermin and insects.
    - Lab workers must report any signs of insects/ pests to the PI or lab manager, who will then contact university Facilities to arrange pest control/removal by appropriate means; lab workers will document any service provided.

4. WORK PRACTICE REQUIREMENTS

4.1 Universal Precautions

Universal Precautions is a method of infection control in which all human/non-human primate (NHP) blood, certain body fluids, fresh tissues and cells of human/NHP origin are handled as if they are known to be infected with HIV, HBV and or other bloodborne pathogens.

The range of tasks performed by personnel who have an inherent BBP risk (and a compelling reason to practice Universal Precautions) can include, but are not limited to:

- Procedures performed on human/NHP blood, serum, blood components or other potentially infectious material/specimens of human/NHP origin, especially those involving needles, scalpels, blades or other sharps.
- Blood draws or finger sticks on humans using hypodermic needles, lancets, etc.
- Research involving the cultivation or manipulation of HIV, HBV or HCV.
- Research using human/NHP cell lines and tissue cultures.
- Biowaste handling, decontamination and disposal.

In any research setting that manipulates biohazardous materials—cultured microorganisms, cell lines, viral vectors, specimen material, recombinant material, biowaste, etc.-- it is recommended that this method become your general approach to handling those materials.

Universal Precautions are summarized below:

- Assume that all blood is positive for HIV, HBV, and HCV.
- Assume that all other human fluids/tissues (as well as other types of biological material) are infectious as well.
- When it's difficult to differentiate, treat ALL fluids (e.g., culture material, etc.) as potentially infectious.
- Assume that all individuals are carrying these disease organisms, and avoid skin contact with blood & other potentially infectious materials, such as in instances of providing first aid, etc.
- Avoid eye, nose and mouth contact with blood and with other potentially infectious materials.
- Avoid punctures/sticks with contaminated sharp objects.

4.2 No Food, Drink Or Smoking

Eating, drinking, smoking and storing food or food/ beverage containers (e.g., coffee mugs, water bottles, etc.) is not permitted in any BSL-1 or BSL-2 lab area. Food and food/ beverage containers must be stored outside the laboratory area in cabinets or refrigerators designated for this purpose.

4.3 No Personal Items In The Lab

- Handling contact lenses and applying cosmetics, even Chapstick, is not permitted in any BSL-1 or BSL-2 lab area to prevent hand-to-face exposure to biohazardous agents.

- Keep personal items away from your lab bench area to avoid the possibility of contaminating them. (e.g., personal cell phones, backpacks, Ipods, car keys, pens, pencils, notebooks, calculators, laptop/tablet/Ipad, coat, hat, etc.)

- Potentially contaminated items such as pens, pencils, notebooks, etc. that you use at your lab bench should remain there—do not take them to an office, your car, your home, etc. to avoid the possibility of fomite transmission of biohazardous agents.

4.4 Hand Hygiene

- Cover cuts or abrasions on hands when in a biohazardous work area.
- Hydrate dry, cracked skin with lotion to improve skin integrity on hands.
- Always keep hands gloved when working with biohazardous agents, making sure your wrists are not exposed.
- Never touch your face with gloved hands.
- Never put your hands in your personal pockets while gloved.
- If you need to access your lab coat pockets while working, train yourself to only access them while gloved, not when ungloved.
- When gloved, never touch any work surface that others touch bare-handed. (e.g., keyboard, computer mouse, pencils/ pens, doorknobs, equipment handles, telephone, faucets, etc.)
- Practice effective hand washing:
    - Wet hands with clean, warm running water and apply soap.
    - Rub hands together to lather soap, washing backs of hands and thumbs, between fingers, around nails; wash wrists up to slightly above area covered by gloves.
    - Scrub for 20 seconds.
    - Rinse hands under clean running water; dry using clean paper towels.

- If no soap or running water is available, use moist disposable wipes to physically remove dirt/debris from your hands, then use hand sanitizer on all hand/wrist surfaces. Wipe hands with paper towels before product dries on skin, then reapply sanitizer and let air dray. Wash with soap and water as soon as possible.

4.5 Use Of Sharps

- Use of sharp objects such as Pasteur pipettes, syringe needles, razors, glass slides, etc. must be utilized only when an adequate, less hazardous substitute cannot be found. Plastic ware must be substituted for glassware whenever possible.

- Needle Use In Labs:
  - Hollow bore or hypodermic needles have been adapted for a variety of purposes in the lab other than for their intended use; if needles must be employed, then make use of a type with safety features that shields the needle from the user to reduce the likelihood of puncture injury.

  - **Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.**

  - Substitute safe alternatives for needles whenever possible.

- Choose safe alternatives to any sharps used in the lab if possible (i.e., use a plastic gel cutter rather than a razor blade for cutting gels) or eliminate the use of sharps when you can.

- Personnel must be trained for safe use and disposal of sharps in BSL-1 and 2 work areas.

- Broken glassware must not be handled directly. It must be recovered using a brush and dustpan, tongs or forceps.

- *Always use a mechanical device (such as forceps) to secure the material when cutting with scalpels, razor blades, etc.*

4.6 Personal Protective Equipment (PPE) USE

4.6.1 Minimum Requirements

While working with biohazardous materials in this laboratory, personnel must tie back long hair and wear:

- Closed-toe shoes
- Street clothing that fully cover the legs
- Disposable or cloth lab coat
- Disposable gloves of appropriate the type for work being done
- If determined by risk assessment, the following may be required:
  - Eye and face protection (e.g., goggles/safety glasses, face shield or other splatter guard),
  - Hair covers
  - Disposable sleeves
  - Shoe covers
  - Respirators

    NOTE: Use of respirators requires enrollment in the Respiratory Protection Program.

4.6.2 PPE Use, Decontamination and Disposal

4.6.2.1 Gloves

- Consider all the hazards your gloved hands may contact during your work session: chemical, biological, radiological, sharps, animals, cryogenic items, heat, and combinations of these. Consult a glove permeation/degradation chart for your preferred vendor (many are online) and select gloves made from material which has the longest breakthrough time for hazard contact, or are specifically engineered to withstand the hazards you may encounter.

- Never use regular disposable gloves for heat or cold protection; use insulated gloves.

- When autoclaving, make sure your insulated gloves are not wet; wet gloves convey heat and will not protect your hands.

- Be sure your gloves fit well for maximum safety—always use the right size of glove.

- Gloves must be changed when contaminated, when integrity has been compromised, or when otherwise necessary.

- Disposable gloves must not be washed or reused.

- Be aware that frequent spraying of gloved hands with a disinfectant can increase permeability to biohazards and chemicals with some glove materials.

- Remove gloves as follows to avoid self-contamination:

  - Pinch one glove at wrist level and peel it off the hand without touching your skin, allowing the glove to turn inside out.

- Holding the removed glove in the gloved hand, and slide fingers of ungloved hand between the glove and the top of the wrist. Remove the second glove by rolling it down the hand, and fold it into the first glove so that it is captured within, and the outer glove is inside out.
- Holding an inside surface of the combined gloves with your bare hand, discard them into solid biological waste. Wash hands immediately.

- Contaminated gloves must NOT be disposed of in regular trash that housekeepers empty. They must be discarded into lab solid waste containers.

4.6.2.2 Lab Coats

- Lab coats must have closures fully fastened to protect from contamination; button your lab coat.

- Ensure that there is no gap between the lab coat cuff and the glove cuff—no exposed skin. Use disposable sleeves if necessary. Tuck the cuff of the lab coat sleeve into glove cuff.

Disposable Lab Coats/ Gowns

- Best suited for higher biosafety level work, especially back-closing gowns.
- Normally discarded after a single use; dispose of in solid biological waste.
- Limited re-use can be allowed, depending on agents/ procedures used, PI discretion, and if the coat/gown is intact, unsoiled and not contaminated.

Cloth Lab Coats

- Best suited for BSL-1 and general bench work.
- If coat is intact, unsoiled and not contaminated, it can be re-used for a period of time determined by the PI.

Cloth Lab Coat Decontamination And Laundering For Re-Use

- BSL-2 cloth lab coats must be decontaminated by autoclaving or other approved method prior to laundering; decontamination by autoclaving is recommended for BSL-1 lab coats.
- Collect soiled lab coats in a dedicated, labeled, lidded container with Biohazard label until they can be decontaminated; line the container with an autoclave bag prior to collection of lab coats.
- Autoclave lab coats in a clear autoclave bag, or label an opaque bag well so contents will not be mistaken for biowaste.
- Decontaminate lab coats using an autoclave Solid or pre-vac cycle; use a Chemical Integrator (CI) for load verification.

- It is strongly recommended that 1) decontaminated lab coats are laundered by a professional service, and 2) that individuals do not take them home to launder them after autoclaving.

4.6.2.3 Eye/ Face Protection

- Persons who wear contact lenses in laboratories must also wear eye protection in the form of tight fitting goggles if their work is not confined to a BSC, chemical fume hood, or behind a bench shield that blocks potential splashes to face.

- Evaluate the splash/droplet potential for exposure to eyes, nose and lips; wear a face shield over safety glasses/goggles to be fully protected.

- Protect eyes from UV radiation by using face shields, safety goggles, equipment eye shields, etc. that have the ANSI Z87.1 safety rating imprinted on the browband or earpiece.

- Pull down the glass sash on the BSC when UV is on to protect eyes.

- Safety glasses, goggles, face shields, etc. must be decontaminated and cleaned after each use prior to storage.

4.7 Storing Biohazardous Materials

4.7.1 Storing/ Holding Cultures on the Bench, in Refrigerators and 4˚C Freezers

- All cultured biological material must be stored in <u>closed containers</u> (e.g., lidded plates, capped tubes, etc.). Lids/ caps can be loosened enough to allow for maintenance of aerobic conditions as needed within the culture vessel, but must not be so loosely placed that they can fall off or be easily dislodged from vessel openings. Culture plates/tubes and their lids may be further secured with a parafilm seal, as needed.

- All stored tubes (e.g., screw-cap tubes, snap cap plastic tubes, eppendorf tubes, conical screw-cap tubes, etc.) containing culture material must be <u>secured in tube racks or boxes</u>. Racks/boxes of culture tubes must not be placed precariously on the bench or refrigerator/freezer shelves; make sure they are solidly placed.

- Stacks of stored culture plates must be secured in <u>plate carriers, pans, or plastic sleeves with taped closures</u> to prevent spillage.

- All stored culture vessels must be <u>legibly labeled</u> with contents and date. If vessels are too small for full labeling, use a coding system and a log or record that coordinates each culture code with: 1) complete identity information for contents, 2) date, and 3) rack or box location of the culture, 4) name of researcher.

- **All refrigerators, freezers and dewars used for biohazard storage must be labeled with the biohazard symbol.**

4.7.2 - 80˚C Freezer Storage

- This type of freezer storage is essential for preserving valuable sample inventories in many labs. Most freezer models are equipped with alarms to warn of systems failure and warming interior temperatures. *If inventory maintenance is critical*, the PI or lab manager needs to implement some means by which personnel can be immediately notified of alarm conditions.

- Keeping a daily temperature log for any -80 freezer is highly recommended for quality assurance purposes, i.e., to monitor the range of daily fluctuations, for malfunctions/ error messages, and for trends of deteriorating performance.

- Hazards: The operating temperature of - 80˚C (-112˚F) is capable of inflicting frostbite very quickly; skin must be protected from direct contact with surfaces at this temperaure, especially metal surfaces, by using insulated gloves.

- Chronic mishandling of these freezers can shorten their useful life and/or result in sudden operational failure, risking loss of irreplaceable samples. The following guidelines are provided to avoid this outcome:

    - - 80 freezers do not automatically defrost; in fact, ice builds up in them at a rate that requires defrosting with some regularity to avoid accumulations that will interfere with door closure. DO NOT FORCE DOOR CLOSURE OVER ICE BUILDUP; THIS CAN DEFORM THE DOOR AND HANDLE, MAKING THE UNIT INOPERABLE. In such a situation, immediate defrosting is called for rather than forcing closure.

    - Ice buildup also can make sample retrieval challenging; be sure your samples are well organized and in well-labeled racks or boxes that are intended for -80 freezer storage.

    - Keep a diagram of exactly where you store materials in the freezer so you can go right to what you need, vs. searching randomly with the freezer door open and contents warming.

    - OPEN FREEZER DOORS FOR THE MINIMUM TIME POSSIBLE, AND FOR THE MINIMUM NUMBER OF TIMES OF DAY POSSIBLE. Coordinate with coworkers to access freezer contents in one or two trips to avoid multiple freezer openings throughout the day. Each opening challenges the cooling capacity of a -80 freezer to recover its set

temperature; the longer and more frequently freezers are open, the more challenge is imposed, which can compromise long-term function.

- Defrosting:
    - Schedule a -80 freezer defrost procedure with plenty of lead time in which to plan where you can relocate your inventory. -80 storage space in labs is usually at a premium, so finding extra space to temporarily house your inventory may be difficult. NOTE: Holding -80 inventory in -20˚ or -4˚ freezer space WILL NOT keep them adequately frozen. If you cannot find temporary -80 freezer space, holding inventory in loosely-lidded Styrofoam boxes with dry ice is an acceptable alternative, if you keep the dry ice well supplied.

    - Expect the entire process to take two days.

    - Wear appropriate PPE for this process. Remove your inventory from the freezer in an organized way so that you can return it to the -80 in good order. Take advantage of this opportunity to cull any inventory that is no longer needed.

    - When your inventory is secure elsewhere, power down the freezer and place a sign on it that warns others: "Out of Service -- Defrosting in Progress – Unit Will Be Decontaminated for Biohazardous Materials by: (your name) Before Return to Service."

    - Prop the door open to let thawing begin; a floor fan blowing room air into the freezer increases thawing rate. Expect complete thawing to take at least 24 hours.

    - DO NOT USE SHARP OBJECTS TO CHIP ICE OUT OF THE FREEZER TO HASTEN THE PROCESS—THIS CAN DO STRUCTURAL DAMAGE TO THE FREEZER. Place pans on the shelves to catch melting ice. If the freezer stores biohazardous material, add one part bleach to 9 parts melted ice, and allow contact time before discarding melt-water down lab sink.

    - Place paper towels on floor to absorb melt-water. As they get wet, discard them in solid biowaste for autoclaving and replace them with dry towels. Decontaminate damp floor area with 10% bleach or other appropriate disinfectant.

    - When ice is gone, decontaminate interior and exterior surfaces of the freezer with 10% bleach or appropriate disinfectant, and allow to dry fully.

    - If accessible, clean dust and dirt off the exterior coils on the back of the freezer.

- Turn power on and allow freezer to come to temperature. Leave freezer empty for at least a day to ensure that it will attain and hold temperature before returning inventory to it. It is common for freezers to fail following an out-of-service period, so monitor carefully.

- Plan the re-installation of inventory carefully so that it can be accomplished in as few sessions as possible, and as quickly and orderly as possible.

- Keep close check on the freezer after inventory has been returned to ensure that freezer returns to set temperature and holds there.

4.7.3 Liquid Nitrogen Storage

4.7.3.1 Health and Safety Hazards

- Liquid nitrogen is extremely cold; it boils at -196°C. Skin can survive brief contact with – 80 surfaces, but bare skin coming into contact with liquid nitrogen (or objects cooled by it or gases evolving from it) will be severely damaged, comparable to burns caused by contact with boiling water. Insulated cryo-gloves will protect you against liquid nitrogen vapor, but will not offer complete protection against direct contact with liquid nitrogen. Skin can freeze or adhere to surfaces cooled by liquid nitrogen, causing tearing upon removal.

- Nitrogen gas sublimating from liquid nitrogen can quickly displace the oxygen in poorly ventilated or closed rooms, and can cause asphyxiation. To reduce the possibility of asphyxiation, use liquid nitrogen only in well-ventilated rooms. DO NOT shut doors when filling containers. If you suspect or encounter a liquid nitrogen leak in your facility, leave the area immediately, alert other nearby personnel, and call for help.

- NOTE: the cloudy vapor that appears when liquid nitrogen is exposed to the air is condensed water vapor, not nitrogen gas. Nitrogen gas is invisible.

- Explosion Hazard: Never place liquid nitrogen (or dry ice) in a sealed container or any object that could entrap the sublimating gas.

- Never mix liquid nitrogen (or dry ice) with water or water ice; never pour it down a sink drain. Ice can solidify around it, trapping sublimating gas at a high pressure and creating an explosion hazard.

- Given the risks associated with the use of liquid nitrogen, best practice is to employ the buddy system when you have to handle this material.

4.7.3.2 Liquid Nitrogen Storage Overview

- **Dewar storage vessels** are vacuum-jacketed tanks for maintaining low temperature storage of biological material; they are designed to safely contain liquid nitrogen as the low temperature agent. Dewars accommodate racks for small sample vials. A loose fitting cap fitting over the neck opening prevents atmospheric moisture from plugging the neck and allows sublimating nitrogen gas to escape. Thus this type of container is non-pressurized. A liquid nitrogen *supply cylinder* is pressurized.

- **Transfer vessels** are designed specifically for containing and transporting liquid nitrogen, i.e., they provide carrying handles, pressure relief valves and venting lids. Only use such a transfer vessel designed for transporting liquid nitrogen to supply a dewar. After filling, a transfer vessel may be carried between two people with its handles, or placed on a cart to transport. If a cart is used, secure the vessel to the cart so it will not tip over when the cart is conveyed over a threshold, etc.

- Always label tubes/vials well for liquid nitrogen storage, and record their placement **and** removal on a dewar inventory log; include tube/vial location within the storage box/can, as well as the designation of the storage box/can. This is best practice because: 1) samples can be efficiently located prior to retrieval, which keeps the time that the dewar has to be open to a minimum; 2) it prevents sample mix-ups, losses, etc.

- Liquid nitrogen must be maintained at a certain volume within the dewar to keep samples at the appropriate low temperature. Levels should never go below 2 inches. Dewars can be outfitted with monitoring alarms which will alert users if the LN2 level drops to a critical point, but these alarms may not be accurate for displaying actual fill levels. The level also can be monitored by immersing a stick reserved for this purpose, to see where the liquid level is detected on the stick. Dewar liquid nitrogen levels should be checked regularly and refilled as indicated. Extra care must be taken and arrangements must be made for holidays/ semester breaks, etc., to prevent depletion when the lab is closed.

- Vapor phase storage is strongly recommended 1) because contamination can be transmitted to submerged vials by liquid nitrogen, and 2) because storing in the liquid phase heightens the potential for explosion of improperly sealed vials when you retrieve the vials to use them.

4.7.3.3 Equipment and Supplies

- DO NOT TRANSPORT OR HOLD LIQUID NITROGEN IN OPEN CONTAINERS, OR IN OTHER CONTAINERS THAT ARE NOT DESIGNED FOR USE WITH LIQUID NITROGEN.

- Storage dewars on wheels must not be rolled on lengthy routes for relocation, or for filling at the liquid nitrogen source. Moving/ jostling the dewar contents could irreparably damage it by cracking the inner metal wall. Moving a partially-to-fully filled dewar also creates a liquid nitrogen spill hazard. Storage dewars must be filled by bringing a supply of liquid nitrogen to them.

- Always use tubes/vials that are recommended for cryostorage; even these products will not withstand prolonged submersion in liquid nitrogen, so be sure to store tubes/vials in the interphase space in the dewar (i.e., in the vapor layer, not the liquid nitrogen layer).

- Always place tubes/vials in cans, canes or boxes that are recommended for dewar storage, or are part of the dewar storage system, before placing them within the dewar.

4.7.3.4 Using a Liquid Nitrogen Supply Cylinder to Fill a Transfer Vessel

1. Always wear eye/face protection, buttoned lab coat, long pants, closed toe shoes and insulated gloves when dispensing liquid nitrogen into a transfer vessel.

2. Hold the filling hose with a secure grip while turning on the tank valve to avoid unpredictable nozzle motion and spillage when flow begins.

3. To prevent splashing, place the filling hose at or below the mouth of the receiving vessel.

4. Slowly turn on the tank valve to begin flow of liquid nitrogen.

5. If flow seems to be mostly vapor and squeals loudly, the tank is almost empty or completely empty.

6. Determining when your transfer vessel is full can be challenging. DO NOT FILL TRANSFER VESSELS UNTIL IT IS OVERFLOWING AS A METHOD OF OBTAINING A FULL TRANSFER VESSEL. Instead, turn off the storage tank valve, remove the hose and check in the vessel periodically to see how quickly it is filling. You will have to wait for the white vapor to clear to see the fluid level. Flow rates for LN2 may not fill transfer vessels quickly, so be patient. Do not fill to very top of vessel.

7. Place lid on the transport vessel before moving. NEVER use a tight-fitting lid on a vessel containing liquid nitrogen.

4.7.3.5 Storing, Retrieving and Reviving Biological Samples in Liquid Nitrogen Dewar

- Maintain a storage dewar in a safe, out-of-the-way location in the lab.

- When inserting or removing racks, be careful not to come into contact with the neck of the dewar; remove or insert racks in a vertical manner to prevent scratching or otherwise damaging this vulnerable area of the vessel. If it is damaged, the vacuum jacket could rupture and ruin the dewar's functionality.

- When accessing the contents of a dewar, always provide yourself with a stable, convenient location to place the dewar top after removing it, as well as safe, stable place to put the ultra-cold storage racks or samples that you remove from the dewar (e.g., lab bench, lab cart surface, etc.).

- Use tongs to remove sample ultra-cold vials from storage canes or boxes. Vials can explode when removed from the dewar, so this should be done in the BSC.

- Always wear eye protection, lab coat, closed toe shoes and insulated gloves when adding or removing samples from liquid nitrogen storage.

- The thawing procedure is stressful to frozen culture material (cell cultures and bacterial cultures). Using good technique and working quickly ensures that a higher proportion of cells survive thawing. Always follow instructions provided with your cells, with reagents used, or in your SOPs for best results when thawing and reviving culture material.

4.7.4 Lyophilization Storage of Biological Materials

4.7.4.1 Storage Overview

- Lyophilization, also known was freeze-drying, is a process in which water is removed from a material after it is frozen and placed under a vacuum. Laboratories performing biological research sometimes 1) utilize lyophilizers for preservation and storage of bacteria, fungi, protozoa, algae, viruses, mammalian tissue cultures or plant cells, and 2) reconstitute cultures in lyophilized form to revive cultured material. Both procedures have inherent hazards. Thus, this equipment and procedures should only be used by persons who have been thoroughly trained.

  - Lyophilized mammalian cells, plant cells, protozoa and algae must be maintained below -150˚C in a liquid nitrogen storage system for long-term stability. Lyophilized bacterial cultures can survive storage at -60˚C for several years, and should never be stored at temperatures greater than 4˚C.

4.7.4.2 Lyophilizer Hazards

- A high vacuum is generated with this equipment which can cause implosion of glass ampules. Safety glasses/goggles/ face shields and other appropriate PPE must be worn at all times when using a lyophilizer.

- Lyophilizers generate low temperatures. Because ampules and other parts of the machine may cause cold burns if touched to exposed skin, protective gloves must be worn when handling these cold parts, or avoid contact.

- There is a risk of electrical shock hazard if a lyophilizer is misused, or is malfunctioning. Inspect for any problems before using a lyophilizer (e.g., problems with power supply, sparks, burning smell, etc). If problems are discovered, do not use the unit and notify the individual who is responsible for it.

- Never use a lyophilizer for evaporating material containing organic solvents.

4.7.4.3 Reviving Lyophilized Biological Material

- The process of liberating lyophilized culture material from an ampule can create aerosols of dry culture material and present a biohazardous exposure risk. Only perform this procedure in the containment of a BSC, and ensure that work surfaces and surfaces of supplies are decontaminated following your procedure.

- The glass ampules used for lyophilized cultures actually must be broken in a controlled procedure to liberate their contents. Due to the significant risk of receiving cuts from broken glass, as well as risk of exposure to biohazards, you must wear appropriate PPE (e.g., hand protection, lab coat, eye/face protection) for this procedure. Use the instructions provided with cultures you have ordered from a commercial provider. For lyophilized cultures produced in house, the following ATCC method can be used:
  1. Score the ampule with a sharp file near the tip of the ampule.
  2. Disinfect the ampule with alcohol-dampened gauze. (Make sure the gauze not too wet.)
  3. Wrap the gauze around the ampule, then using two hands, break the ampule at the scored area. Take care that alcohol from the gauze is not sucked into the dry culture material when the vacuum is released.
  4. Rehydrate the material at once.

4.8 Transporting Biological Materials

4.8.1 Moving Biohazardous Materials from One Work Area to Another

- **Transport outside of the laboratory but within the building** -- Place within a durable, leak-proof container, such as a plastic sterilite box plus lid; close lid and disinfect the outside

container before removing from the room. Either the primary or the secondary container must be labeled with the biohazard symbol.

- **Transport outside the building** -- Seal the material in a primary tube/flask or other leak-proof container; place biohazard symbol on this container. Disinfect the container before placing in a durable, leak-proof, lidded secondary transport box or container. The secondary container will be disinfected and securely closed for transport.
- For added safety, containers should be transported on a cart to further minimize spill hazards.

4.8.2 Transporting Biohazardous Waste to Autoclave Facilities

- Waste must be securely closed and sprayed thoroughly with 70% ethanol.
- Waste must be placed within a secondary container (e.g., Nalgene or stainless steel pan, plastic lidded tub, etc.) which is dedicated for this function and labeled with a Biohazard label.
- Waste must be transportedPlace in secondary containers on a cart to autoclave facilities.

4.9 Shipping Biological Materials To Domestic And International Destinations

- Contact EHS Biosafety group if you need to ship biohazardous materials with or without dry ice.
- Make contact at least 3 working days before your preferred shipment date; **allow longer for international shipments**. These shipments are typically mailed on Monday - Wednesday.
- A certified shipper of hazardous materials at EHS with work with you to prepare for the shipment, as follows:
  - The sender completes a Biohazardous Shipping Request Form and submits it to the EHS certified shipper, who 1) determines how the shipment must be classified according to biosafety level, presence of chemicals/preservatives, etc., and 2) schedules a shipment date with the sender.
  - EHS certified shipper discusses these specifics with the sender: 1) sender must obtain needed permits and provide them to the EHS certified shipper prior to shipment; 2) sender must provide cold packs or dry ice in appropriate quantity, if needed, at time of packaging for shipment.
  - EHS certified shipper will determine and provide the appropriate packaging system, attach required labels/ markings to outer package, and prepare airway bill and other needed documents.
  - EHS certified shipper arrives at sender's research laboratory on agreed-upon date, and works with sender to package the shipment. The certified shipper will take the sealed package to EHS for pickup by the carrier.

4.10 Biological Waste Management

4.10.1 Contaminated Waste Items Generated In A Biosafety Cabinet

- Items may be removed from the BSC after they are decontaminated with an appropriate disinfectant, such as being sprayed with 70% ethanol, or they can be placed within a bag or sealed container in the BSC, which is sprayed with disinfectant before removal from the BSC.

4.10.2 Liquid Biohazardous Waste

- Preferred Method For Liquid Biowaste Containing No Disinfectant Or Other Chemical Components:
  - Collect the waste in a well-labeled autoclavable container (containing no bleach), then autoclave the waste on a liquid cycle at 121˚ C, with the sterilization time determined by the liquid volume. After cooling, the waste may be poured down the lab drain.

- Alternative Method:
  - If using the above method is not possible, liquid biowaste can be discarded into a container containing a sufficient quantity of bleach (e.g., pure bleach to yield a 1:5 dilution. (Example: 100 mls. household bleach added to 400 mls. tissue culture media).
  - After the required exposure time in the BSC, the liquid waste plus bleach may be disposed of down the lab drain, followed by a water flush. If a different disinfectant is used, it must be disposed of as chemical waste.
  - Decontamination by bleach a less reliable decontamination method because of the opportunities for a failed result if the bleach used is 1) expired, 2) present in the wrong proportion to liquid waste volume, and 3) not given sufficient contact time before disposal.

- Liquid Biowaste Containing Bleach, Another Chemical Disinfectant, Or Other Chemical Constituents Such As Sodium Azide:
  - Dispose of as liquid chemical waste. DO NOT AUTOCLAVE THIS WASTE.

- Liquid Biowaste Containing Antibiotics:
  - Media wastes containing the following antibiotics can be autoclaved, cooled and flushed down the lab sink drain because the antibiotics are broken down by heat, and are then environmentally safe to go into the domestic sewer.
    Heat-sensitive antibiotics:

Ampicillin	Kanamycin
Amphotericin	Neomycin
Carbenicillin	Puromycin
Penicillin	Streptomycin

Geneticin Tetracycline
Gentamicin

- Media wastes containing the following heat-stable antibiotics cannot be autoclaved and discarded into the domestic sewer because of their relatively long half-life which persists in the environment; add bleach in a 1:5 vol/vol concentration to deactivate biologicals, then dispose of as liquid chemical waste.
  Heat-stable antibiotics:
  Hygromycin B
  Chloramphenicol
  Ciprofloxacin
  Vancomycin
  Nalidixic acid
  Zeomycin

- If antibiotics are used that are not on these lists, contact EHS for disposal consultation, or simply submit for disposal as liquid chemical waste.

- **Media with additives such as growth factors, metals or other chemicals must be disposed of as liquid chemical waste.**

- Liquid decontamination method(s) should be verified and documented.
- Liquid biowaste containers must be appropriately labeled as such, with biohazard signage; liquid chemical waste containers must be appropriately labeled and well-identified as a different waste stream.

- Liquid Wastes Containing Extracted DNA/ Cellular Components/ Lysates, etc.

  - Biological material treated with extraction kit chemicals or other chemical treatments that lyse cell membranes renders bioagents non-viable and no longer hazardous. This material must be disposed of as chemical waste.

4.10.3 Solid Biohazardous Wastes

- BSL-1: Collect in a non-colored autoclave bag with no Biohazard symbol.
- BSL-2: Collect in an ORANGE autoclave bag with a Biohazard symbol.
- Autoclave bags must be kept inside appropriately labeled biowaste containers, equipped with a closeable lid.
- Solid biowaste containers must remain closed except when in use.

- At end of a work session or when bag is 2/3 full, securely close the bag, place it in a secondary container (Nalgene or stainless steel pan) reserved for this purpose, and spray it with appropriate disinfectant prior to transport on a cart to the autoclave room.

- Proceed with autoclaving the waste according to standard procedures provided in Section 4.14. Bags of solid biowaste must be autoclaved on a gravity or pre-vacuum cycle at 121˚ C; the length of cycle used must be determined by the size and density of the autoclave load. Each load must be checked with a verification device to confirm that kill conditions were met.

- Bags must be autoclaved daily when possible, or as soon as an autoclave is available. No full bags should be left in the laboratory or the autoclave/glassware room OVER WEEKENDS OR BREAKS.

- Bags must remain closed *until they are ready to be autoclaved, at which time their closures should be loosened to at least a 1-inch opening to allow steam penetration.*

- All BSL-2 solid waste is to be decontaminated by autoclaving and disposed of as Regulated Medical Waste.

- Decontaminated BSL-1 waste can be discarded into regular trash, or disposed of in Regulate Medical Waste (see Section 4.8.3).

- Agar Plates With Antibiotics In The Medium:

    - BSL-1 -- It is acceptable to autoclave BSL-1 waste containing heat-stable or heat-labile antibiotics, and once decontaminated, dispose of waste in dumpster trash bound for landfill. Bagged waste is well contained, allowing adequate time for breakdown of antibiotics when disposed of in a landfill.

    - BSL-2 – It is acceptable to autoclave BSL-2 waste containing heat-stable or heat-labile antibiotics, and once decontaminated, this waste must be discarded in Regulated Medical Waste---the same disposal method as BSL-2 plates containing no antibiotic.

- Solid Biowaste/ Lab Debris Mixed with Chemical Waste:
    - Contact EHS to determine the best disposal method.

4.10.4 Sharps Biohazardous Wastes

- Sharps contaminated with biohazardous materials include anything that could puncture an autoclave bag. Examples include:
    - Pipette tips
    - Wood applicator sticks/ swabs
    - Syringe + needles

- Blades
- Glass slides/cover slips
- Serological pipettes
- Glass Pasteur pipettes
- Disposable plastic pipettes
- Broken glass
- Blood tubes/capillary tubes

- Discard all sharps used with biologicals into lidded, rigid, labeled Bio-sharps containers. EHS supplies containers that meet the requirements of being closeable, puncture-resistant and leakproof; they can be requested through the EHS online Safety Management System.

- Sharps waste containers:
  - Must be located for ease of accessibility, i.e., at or near point-of-use.
  - Must be replaced routinely
  - Must be securely closed before removal from point-of-use
  - Must have NO chemical or liquid waste placed within (with the exception of specimen tubes containing blood)

- Never re-cap needles or scalpel blades before disposal into a sharps container.

- Avoid forcing sharps waste into a full container; this can cause puncture/ cut injuries.

- All sharps containers are to be autoclaved using the same cycle as used for biological solid waste and then disposed of in the same manner as BSL-2 waste, i.e., in Regulated Medical waste.

- In situations when collection of Sharps waste is intermittent or of smaller volume, avoid collecting them in large sharps containers. Use smaller Sharps containers whenever possible, as these will fill more quickly and thus can be decontaminated more expeditiously.

- Rigid plastic laundry detergent bottles with lids can be used but must be well labeled.

4.10.5 Regulated Medical Waste (RMW)

- RMW collection boxes are used for end-point disposal of DECONTAMINATED (i.e., autoclaved) biological Sharps and ALL BSL-2 solid waste.

- Decontaminated BSL-1 waste can be disposed of in regular trash cans lined with black bags, or in RMW.

- RMW must not contain any liquids in containers, or free liquids.

- RMW Box Assembly and Use:
  - Tape bottom center seams 2-3X, and tape open side seams one time with wide cellotape.
  - Line each RMW box with 1-2 red RMW plastic liner bags. If using 2 bags, place one inside the other. *Remember, these liners cannot substitute as autoclave bags—they disintegrate in high heat.*
  - Do not overfill RMW boxes, or pack them so that they are too heavy; limit is 50 lb.

- RMW Final Packaging:
  - When a RMW box is full, twist the top of the inner bag enough times so that you can loop it over on itself in a "gooseneck."
  - Seal the twisted top by wrapping with tape. Seal a second, outer bag by the same method.
  - Close the box's top and seal X3 on center seam with wide cellotape, then tape the open side seams 1X.
  - Fill out the white label for the box that identifies the generators of the waste, and the date the box is closed and sealed. Be sure to list all labs that contributed waste to the box. Apply the label in the designated area on the box.
  - Be sure you write the date of closure on the box, not the date the box was assembled and began to be used.
  - Notify EHS when you have RMW waste for pickup using the EHS online request system.

4.10.6 Animal and Animal-Related Wastes

- Lab workers who produce a minimal volume of biological material with no chemical present (e.g., tiny pieces of dissected unfixed tissue) can dispose of that material in solid biowaste which will be autoclaved.

- Lab workers who produce larger volumes of this waste or other types of animal/animal-related wastes should refer to Virginia Tech's Animal and Animal-Related Waste Procedures charts to determine proper waste handling and disposal. Categories of wastes included in the charts are:
  - Animal tissue with/ without fixative or other chemicals present

- Animal tissue or carcasses with/ without hazardous biological agents, rDNA, etc.
- Companion animal carcasses
- Related wastes: bedding, disposable containers, fecal material, dressings, etc.
- Associated sharps and liquids
- Blood collection tubes
- Radioactive wastes

4.10.7 Plant and Plant-Related Wastes

- BSL- 1P, BSL2-P Greenhouse Plant Waste
    - Examples include transgenic, exotic, or infected plant material; soil; pots; etc.
    - Collect in clear autoclave bags or other bags/containers appropriate for the method of biological deactivation to be used.
    - Heat inactivation can be accomplished by:
        - Autoclaving (for smaller volumes) at 121˚ C, 15-30 psi, for 15-180 minutes, depending on type and state of material.
        - Treatment in greenhouse steam box/soil sterilization box (for larger volumes, for fungal, viral or nematode plant pathogens under permit) at ≥ 104˚ C for 3 hours. NOTE: Permission to use greenhouse steam box must be obtained from the greenhouse manager.
    - BSL-1P material can be composed or desiccated, according to IBC approved protocols.
    - Deactivation of plant/seed material must be confirmed before disposal. Acceptable methods of confirmation include:
        - Recording time/temperature criteria of heat treatment used.
        - If autoclaving, use verification devices.
    - Disposal:
        - Following successful deactivation, material can be discarded in regular trash cans with black liners.

- Plain Plant Waste
    - Non-transgenic, non-exotic, non-infective laboratory plant waste is still laboratory-generated waste, and can appear identical to other more hazardous plant wastes.
    - According to your lab-specific situation, this type of waste can be handled in several ways, at the PI's discretion:
        1. Collect, deactivate and discard this waste as if it is BSL-1P or BSL2-P waste.

2. Collect material in opaque trash bags; when full, close securely and discard bags in dumpster.

4.11 Disinfection Agents

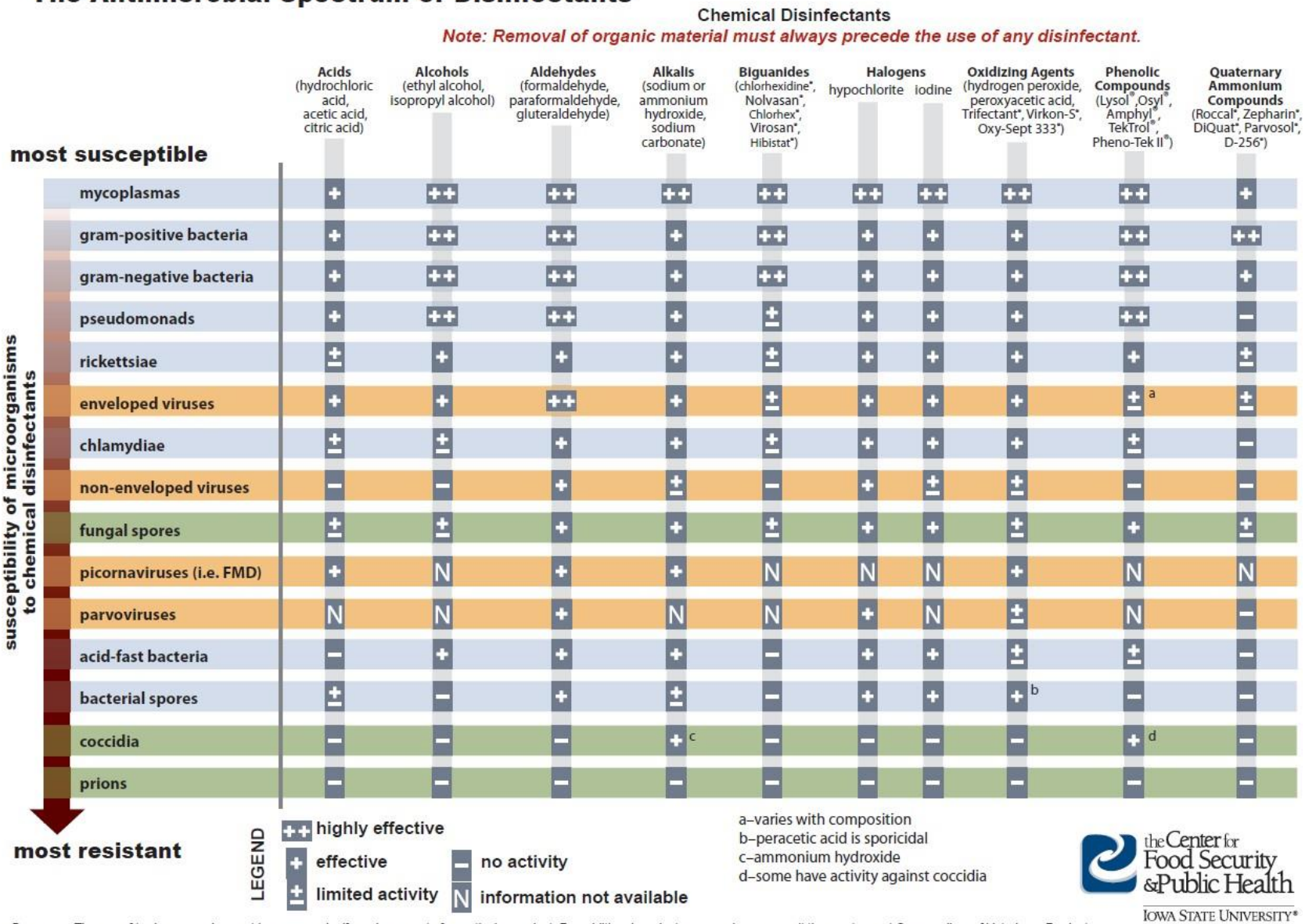
4.11.1 Choosing A Disinfectant

Choose the most effective disinfectant(s) for your lab based on the following criteria:

- The type of biohazardous materials you are working with (fungal/ bacterial/ vegetative vs. spore formers, etc.) and their risk assessments.
- The degree of contamination you typically encounter.
- Whether organic material is/could be present which reduces effectiveness of some disinfectants.
- How the disinfectant works chemically, and in what quantity and concentration.
- What contact time and temperature is needed for disinfectant to be effective.
- How the disinfectant affects materials, such as corrosiveness, or leaving a residue on surfaces.
- What environmental impact it has, such as toxicity, creating noxious fumes, or being an irritant for the user.
- What sort of shelf life it has.
- How expensive it is.

Labs working with different biohazardous materials may find it necessary to stock several disinfectants to supply effective decontamination for all agents. See the following chart for general information on common types of disinfectants used in labs.

The Antimicrobial Spectrum of Disinfectants



Disclaimer: The use of trade names does not in any way signify endorsement of a particular product. For additional product names, please consult the most recent Compendium of Veterinary Products.

Adapted from: Linton AH, Hugo WB, Russel AD. Disinfection in Veterinary and Farm Practice. 1987. Blackwell Scientific Publications; Oxford, England; Quinn PJ, Markey BK. Disinfection and Disease Prevention in Veterinary Medicine, In: Block SS, ed., Disinfection, Sterilization and Preservation. 5th edition. 2001. Lippincott, Williams and Wilkins: Philadelphia.

Source: http://www.cfsph.iastate.edu/pdf/antimicrobial-spectrum-of-disinfectant

4.11.2 Bleach

- Bleach dilutions are inactivated by organic matter and are corrosive to metal surfaces and the skin.
- Contact with bleach can degrade disposable gloves.
- Never use bleach in the presence of formaldehyde.
- Never mix bleach with ammonia or acidic body fluids as toxic chlorine gas will be released.
- Bleach disinfectant solutions must be **made up fresh weekly** as the effectiveness of sodium hypochlorite decreases rapidly with time.
- **Expiration dates of undiluted bleach and all other disinfectants must always be checked; up-to-date stocks must be maintained in the laboratory.**
- In the U.S., bleach designated for general purpose or household use was previously formulated between 5.25% and 6% (industrial strength bleaches are often formulated at concentrations greater than 20%). Leading manufacturers such as Clorox® are now producing household bleach at 8.25%

Using Bleach as a Disinfectant

Purpose	**Dilution of Standard Household Bleach** (min. 5.25% sodium hypochlorite)	**% Sodium Hypochlorite (NaOCl / ppm)**	**Precautions**
Spills of material with large amounts or concentrations of organic matter (e.g., blood), liquid media	1:5 dilution 1 part bleach + 4 parts water or contaminated liquid (e.g., 20 mls bleach + 80 mls water/media/contaminated liquid)	1% NaOCl (10,000 ppm)	• Bleach-based disinfectants can cause skin, eye and lung irritation. Always wear appropriate PPE. • Skin and eye protection must be worn when handling undiluted bleach solution. • Make sure you are in a well ventilated area. • Bleach is corrosive to metal so all surfaces should be rinsed with water following contact with bleach.
Surfaces with large amounts or concentrations of organic matter	1:10 dilution 1 part bleach + 9 parts water (e.g., 10 mls bleach + 90 mls water)	.5% NaOCl (5,000 ppm)	
Surfaces with low amounts or concentrations of organic material	1:50 dilution 1 part bleach + 49 parts water (e.g., 2 mls bleach + 98 mls water)	.1% NaOCl (1000 ppm)	

4.11.3 Ethanol / Isopropanol

- The disinfecting ability of ethyl alcohol and isopropyl alcohol drops sharply when either is diluted below 50%, or at dilutions higher than 90%. Optimum disinfection occurs at 70% in solution with water. Reason: Alcohol's mode of action as a disinfectant is protein denaturation, and water supports the denaturing of proteins. Because pure alcohol is very dehydrating to microbial cell walls (which can interfere with protein denaturation) the presence of a certain amount of water in alcohol more readily denatures microbial proteins.

- Avoid spraying alcohols on surfaces too thinly, resulting in quick evaporation and not enough contact time to achieve disinfection.

- Frequently spraying disposable gloves with alcohol can increase their permeability to biological agents, as well as degrade gloves.

4.12 Decontamination Of Work Surfaces

- All work surfaces (e.g., in biosafety cabinets, bench tops, etc.) must be decontaminated/ disinfected immediately after work is complete and after any spill.

- Surfaces in the lab such as computer mouse and keyboard, etc. that are commonly used with ungloved hands are nevertheless potential sources of exposure, and should be decontaminated on a regular basis, i.e., at least weekly.

4.13 Lab Equipment Decontamination Requirements

- Equipment used with potentially infectious material must be decontaminated:
  - Routinely (e.g., daily or weekly depending on frequency of use)
  - After any spill or splash, and
  - Before any repair, maintenance or removal from the lab.

- If equipment failure or a spill occurs during use of a BSC with biohazardous materials, the work surfaces must be disinfected, and the cabinet's fan, filters and airflow plenums may need to be decontaminated by formaldehyde gas or another approved method. Contact the EHS Biosafety Office for assistance.

- Equipment failure experienced with refrigerators, freezers, water baths, centrifuges, etc. which are used with biohazardous materials also require decontamination and cleaning before being serviced and put back into use.

- Decontaminate refrigerated equipment before having interior surfaces exposed for defrosting. Document those actions using a written notice and post the notice on the equipment while defrosting is in process.

- Decontaminate and clean equipment scheduled for service or surplus, as well as the areas around/ behind/ underneath the equipment that may have accumulated dirt or contaminated debris since equipment was last moved. See instructions on *Equipment Decontamination Form* for approved decontamination methods. (Form can be found in the Appendices of this manual.) Document your decontamination method on the form, and affix the form to the equipment upon completion. For liability purposes, a copy of the form must be kept in lab records following service on equipment.

4.14 Autoclave Use

4.14.1 Hazards

- Potential safety risks for autoclave users include:
    - Burns from touching hot autoclave chamber surfaces
    - Steam burns from residual steam when door is opened at end of cycle
    - Hot fluid scalds from boiling or overboiling liquids/ spillage
    - Hand and arm injuries related to door operation
    - Explosion of the pressurized chamber could injure or kill persons nearby
    - Inhalation or other exposure to fumes/vapors from chemicals that should not have been autoclaved
    - Exposure hazards from handling biohazardous materials before autoclaving, or after autoclaving if material was unsuccessfully decontaminated

4.14.2 Training Requirement

- Successful completion of autoclave training (EHS online module *Safe Autoclave Use* and hands-on training in your facility) is required prior to operating an autoclave at Virginia Tech due to the serious hazards associated with use of this equipment.

4.14.3 Overview of Autoclave Operation

- Autoclaves are essential for bioresearch, providing a reliable means for sterilizing equipment and supplies, and for decontaminating biohazardous waste. Autoclaves are designed to accomplish this by applying steam to items in a pressurized chamber; steam penetration and actual surface contact with the steam is required for sterilization/decontamination of materials to take place.

- Examples of lab materials that can be autoclaved: metal items, glass items, heat-resistant plastic items (e.g., Nalgene, polypropylene, polycarbonate), pipette tips, aqueous solutions, water, animal food and bedding, soil, biohazardous waste.

- Biohazardous waste types to be decontaminated by autoclaving:

  - Solid waste in autoclave bags -- gloves, paper towels, empty tubes, agar media in Petri dishes, other solid lab debris contaminated with biohazards.
  - Liquid biological waste: exhausted culture media, supernatants, etc.
  - Sharps waste in rigid containers: glass slides, needles, syringes, pipettes, tips, blades, etc.

- Examples of items that must not be autoclaved: chemical solvents, corrosives, flammable liquids, other chemicals or chemical containers, kit chemical bottles, antibiotic bottles, vials or tubes with chemical residue, radioactive material, any sealed container.

- Longer sterilization/decontamination times are needed as load sizes/densities/volumes increase.

- **The Solid/Gravity cycle** supplies steam to the chamber with no mechanical vacuum assistance, i.e., by gravity. When process time is complete, steam is quickly exhausted from the chamber. Items such as upright containers that trap air within them cannot be fully sterilized/ decontaminated using this cycle because the trapped air prohibits full steam penetration and surface exposure.
  - Use the Solid/Gravity cycle for: Pyrex/borosilicate glassware (empty, inverted, no closures), dry hard items (unwrapped or in porous wrap), metal items with porous parts, other porous materials.
  - Use the Solid/Gravity cycle for decontamination of solid biological waste if you have no Pre-vacuum cycle available on your autoclave; compensate for poor steam penetration in the waste by lengthening the process time as needed.
  - Do not use this cycle for: liquids or media that require a slow exhaust.

- **The Liquid cycle** also supplies steam to the chamber with no mechanical vacuum assistance. When process time is complete, steam is slowly exhausted from the chamber to prevent boil-over of liquids. NOTE: Because this slow exhaust phase takes extra time to complete, plastic materials that will withstand fast-exhaust cycles can melt using the Liquid cycle due to prolonged exposure to heat.
  - Use the Liquid cycle for: Pyrex/borosilicate glass containers up to 2/3 full of liquid (liquid media, aqueous solutions, liquid biowaste)
  - Do not use this cycle for: items that could melt during slow exhaust.

- **The Pre-vacuum cycle** mechanically removes air from the chamber, then supplies steam to the chamber in a series of pulses which allows full steam penetration of dense materials. Thus it is the preferred cycle for decontaminating solid biological waste. When process time is complete, steam is quickly exhausted from the chamber.
  - If available on your autoclave, use the Pre-vacuum cycle for: decontamination of solid biowaste and sharps biowaste; sterilization of glassware that must be processed in an upright position, and other dry items that can trap air (e.g., pipette tip boxes)
  - Make sure that bags containing agar plates have only a one-inch opening so that the bag can properly contain melted agar during processing.
  - Do not use this cycle for: liquids, media, lighter weight plastic containers, dry items that will collapse in a vacuum.

4.14.4 Things to Check Before You Autoclave a Load

- Wear required PPE to protect yourself from burns through direct contact with autoclave surfaces when loading items, and contact with hot items, splashes, etc. when removing loads from autoclave: buttoned lab coat, closed-toe shoes, heat-resistant gloves, safety glasses/ goggles.
- Use heat-resistant packaging material and containers.
- Label items to be autoclaved so you can identify them in case they are removed after cycle completion and placed elsewhere.
- Make sure the autoclave drain is clear of debris.
- Check to see if the autoclave is functioning properly (no ABORT message, etc.).
- Include a verification device with every waste load (see Section 4.13).
- Adjust closures on bags of solid waste so they have at least a one-inch opening.
- Check to see that closures on liquid-containing vessels are loosened.
- Arrange items in the load so they are spaced evenly, allowing room between items (no stacking/crowding).
- Make sure containers of liquids are no more than 2/3 full.
- Load items so that they do not touch sides or top of autoclave chamber.
- Properly place bags of waste and liquid items in heat-resistant pans or trays, e.g., bags do not overflow the pans; vessels of liquid are in a pan deep enough to contain potential boil-over.
- Do not over-pack load items into the autoclave chamber.
- Make sure that items for sterilization are being processed in separate loads from waste loads for decontamination.

4.14.5 Response to Malfunctions

- If you see an error message, evidence of an aborted cycle or other operational problems, always record your observations on the autoclave user log, and promptly report them to the person responsible for the autoclave.
- If you discover that the autoclave is dysfunctional, and/or if you think it is unsafe to use, immediately post an 'Out of Service' sign on the unit and report it to the person responsible for the autoclave. Never attempt repairs on a malfunctioning autoclave.
- If you feel an emergency situation is developing or has developed, immediately remove yourself and others from the area and report the situation to the appropriate authorities/emergency response providers.

4.14.6 Standard Pre-Run Procedure:

1. Sign in on autoclave use log.
2. For a waste load, put on disposable gloves and place a verification device into proper position within the waste, (or in an empty, like-sized container if running a liquid waste load). *Only one bag/ container per load needs a device placed in it. After placing the device, adjust bag openings to approximately one inch to allow steam penetration.*
3. Wearing appropriate PPE for burn protection, place load into chamber.
4. Close chamber door and ensure that it closes completely.
5. Select and start cycle.
6. Ensure door has sealed and cycle is successfully underway before leaving the autoclave facility.
7. Set a personal timer to remind you when your cycle will end.

4.14.7 Standard Post-Run Procedure:

1. Ensure that cycle is completely finished and chamber pressure is zero before proceeding.
2. Wearing PPE, open autoclave door and stand aside to avoid contact with any escaping steam.
3. Allow load to cool somewhat with door open; let liquids cool in autoclave for at least 15 minutes.
4. Wearing PPE, carefully remove load from chamber. If liquids begin to boil over, stop and allow more cooling time before removal.
5. Close autoclave chamber door when finished.
6. If running a waste load, check the verification result. Results of autoclave verification must be documented in a BI log (BI results), or on the Autoclave Use log sheet (CI results).

4.15 Verifying Autoclave Performance

4.15.1 Verification Requirement

- Autoclaves used for decontamination of biohazardous waste must be regularly tested to verify their performance in reaching proper decontamination conditions. This is necessary to ensure that infectious agents in waste are inactivated prior to leaving the university for final disposal.

4.15.2 Overview of Testing Methods

- Approved testing devices include Biological Indicators (BIs), and Chemical Integrators (CIs). So-called "autoclave tape" reacts to exposure to heat with a light-to-dark color reaction, but it is NOT a

definitive indicator of decontamination conditions having been met. This tape must not be used as a verification testing method.

- Location of the test BI or CI in a waste load is important. Placement of the test device on the outside of an autoclave bag of solid biological waste will not yield the information needed about conditions on the inside of the bag. Instead, the test device must be placed within the bag, preferably in the center of the waste where steam will have the greatest penetration challenge, but not so deeply buried that it cannot be safely and successfully retrieved after decontamination in the autoclave.

- Depending on the size of your autoclave chamber, a 'load' of waste in need of decontamination may consist of multiple items (e.g., several bags of waste, or a bag and several sharps containers, etc.). You only need to use one test device per load, not in every bag or container in the load. NOTE: The density and volume of your load must determine the length of the cycle you use, i.e., denser, larger loads require longer process times.

- When decontaminating liquid biological waste, place the test device in an empty vessel similar or identical in size to the vessel(s) containing the liquid waste (e.g., bottle, flask, etc.) and cap the vessel with the same type of closure as used on the waste container(s). Prior to autoclaving, place the waste vessels and the vessel with the test device in a secondary container (e.g. Nalgene pan, stainless steel pan), spacing them as evenly as possible and leaving room between vessels to allow for good air flow around them.

- BIs are used to test autoclaves for:
    - Routine performance verification
    - Following autoclave installation and repair
    - When new autoclave cycles are added, or when cycle parameters are changed
    - When a new load configuration or packaging material is introduced

- BIs require incubation to determine results, but they are the more accurate testing method. CIs are not as absolutely accurate as BIs; however, they are generally quite reliable, and provide immediate results (no incubation required). They also are less expensive. Thus, an autoclave verification program which includes both testing methods can make use of each to its best advantage:
    - Verify autoclave performance monthly with BIs and record results in BI log.
    - Verify decontamination of each waste load with CIs and record results on autoclave use log.

4.15.3 Standard Procedure for BI and CI Verification Testing

Biological Indicators (BI)

1. Label each BI with date, autoclave identification and the cycle type you are testing.
2. Place the BI inside a bag of solid biological waste.

3. Label one extra BI as a positive control for the lot number being used. This BI will not be autoclaved; it will be activated and incubated to confirm that the bacterial spores of the test organism will germinate under favorable conditions.
4. Run the autoclave cycle.
5. Fill out the monthly autoclave verification log.
6. Upon cycle completion and cooling of the load, retrieve the BI.
7. Wearing appropriate PPE, follow product instructions to release the culture media in all test vials to put it into contact with the bacterial spores.
8. Make sure the vial incubator is turned on and set at the recommended temperature. Place vials in incubator.
9. Observe and record any color changes at 24 and 48 hours. The positive control should grow (and vial liquid should change color); test vials that have been exposed to successful decontamination conditions in the autoclave should not grow (and vial liquid will not change color).

Chemical Integrators (CI)

1. Check expiration date on CI before use.
2. Wearing appropriate PPE, place a CI within a bag of waste, then adjust the closure of the bag to approximately a one inch opening. If autoclaving liquid waste, place CI in a like-sized container.
3. Run the load on an appropriate cycle for the waste type.
4. When cycle is complete and load has been removed and cooled, retrieve the CI, interpret the reaction according to information supplied with the CI product.
5. Record results on Autoclave Use log.

4.15.4 BI or CI Failure to Verify

- When BIs or CIs indicate decontamination conditions were not met, an investigation of autoclave performance must take place; the machine must be taken out of service until its status can be determined, and malfunction corrected.

- Waste autoclaved with a verification device that failed to validate kill conditions must not be discarded, but re-autoclaved in a different autoclave, or held until the cause of the problem is identified and it can be determined that the waste was in fact sufficiently decontaminated.

- Possible causes of BI or CI failure to verify (other than autoclave malfunction):
  - Test devices “Use By” dates have expired.
  - The wrong kind of test device was used (e.g., a test device designed to test dry heat or gas sterilization equipment); the correct test device will specify that it is meant to be used for steam autoclaves.

- CIs which have been overexposed to light, completely soaked with water, or crimped/bent during use in an autoclave run can result in their performance being compromised.
- Steam did not fully penetrate the load because 1) the bag was packed too densely with material; 2) the bag was not opened at least one inch before the decontamination cycle was run; 3) the autoclave chamber itself was packed too densely which prohibited steam penetration.

4.16 Biosafety Cabinets (BSC)

4.16.1 Overview

- BSCs are specialized pieces of lab equipment designed to safely contain biohazardous agents/ materials and protect sterile items and culture materials from contamination when they are manipulated in the BSC work space. There are several different classes and types of BSCs to meet specific needs. *Choosing the right BSC for purchase should be based on a thorough risk assessment of all material (biological and chemical) being handled and the procedures involved with the work.*

- BSCs must not be confused with chemical fume hoods, which utilize directional air flow to protect lab workers from exposure to toxic chemical fumes or particulates by venting them to the outside. Material in a chemical fume hood is not protected from room air contamination.

- Minute amounts of volatile chemicals/ radionuclides may be used ONLY with certain types of BSCs which exhaust them to the outside. *However, chemical fume hoods should never be used for protective containment of biologicals.* In most cases, BSCs and chemical fume hoods have distinctly different functions and are NOT interchangeable.

- The HEPA-filtered directional air flow in a BSC 1) protects the work material from contaminants, 2) protects the worker from exposure to aerosols, and 3) prevents release of aerosolized material into the environment. IMPORTANT: BSC HEPA filters do not entrap or filter chemical vapors or gases; they entrap particulates.

- The **HEPA filter** is the most important feature of the BSC, capturing potentially infectious particles from your work as well as room air contaminants, and contaminants that you may shed. HEPA filters should be replaced every 3-5 years, depending on cumulative hours of operation, the cleanliness of the lab, and the materials being used in the BSC. Changing or cleaning the pre-filter on a regular basis extends the life of a HEPA filter.

- The **magnehelic gauge** on the BSC shows the air pressure difference across the HEPA filter, and indicates whether the air flow system and filter are operating properly. BSC users should know their BSC's acceptable gauge readings and limits; ask your service provider, or look on the BSC certification label for this information. Before each work session, check the gauge and look for changes higher or lower than this acceptable range. A higher resistance reading indicates the filter is loaded or blocked; a lower resistance reading may indicate a hole or tear in the HEPA filter. In either instance, do not use the BSC; contact a service provider.

- Room location is important to the proper functioning of a BSC:
    - Ideally, BSCs should occupy lab space that is removed from other work areas, especially high traffic areas.
    - Cabinet should be placed 12-14 inches from ceiling and walls.
    - Cabinet should be placed away from windows, air supply vents, lab features creating air movement (chemical fume hoods, centrifuges, vacuum pumps), and entry points into lab.

4.16.2 BSC Classes and Types

SIDE-BY-SIDE BSC CLASS COMPARISON

Classes of Biosafety Cabinets	Personnel Protection	Product Protection	Environmental Protection	Use
Class I	Yes Inward air flow through sash opening	No Unfiltered room air is drawn across work surface	Yes Exhaust air is HEPA-filtered	• Not in use today for bioagents • May be used to enclose equipment or procedures with aerosol potential
Class II A1, A2, B1, B2	Yes Inward air flow through sash opening	Yes By HEPA filtered air drawn down onto work surface & room air kept away	Yes Exhaust air is HEPA-filtered	• *Most common class of BSC used today, esp. Type A2* • Used to handle specimen material, biological toxins, cell tissue culture, biohazardous agents
Class III (Glove Box)	Yes Complete containment of interior work area	Yes HEPA filtered air is supplied to work surface; total containment keeps room air out	Yes Exhaust air is double HEPA-filtered	• Provides the highest level of containment for handling the most dangerous microorganisms

SIDE-BY-SIDE COMPARISON OF BSC CLASS II TYPES

BSC	HEPA-Filtered Work Surface Air	Interior Design	Air Inflow Rate	Chemicals
II, A1	ALL is RECIRCULATED	May have contaminated air under POSITIVE pressure, so if plenum leaks, contaminants will escape into lab	75 linear feet per minute (lfpm)	Use with biologicals; Should NOT be used with chemicals
II, A2	MOST or ALL is RECIRCULATED	Contaminated air under/surrounded by NEGATIVE PRESSURE; if outside exhaust is present, uses flexible connection	100 lfpm	Use with biologicals; recommended for use with MINUTE amounts of volatile chemicals if some air is exhausted outside
II, B1	MOST is EXHAUSTED OUTSIDE	Contaminated air under/surrounded by NEGATIVE PRESSURE; outside exhaust must be hard-ducted	100 lfpm	Use with biologicals & MINUTE amounts of volatile or toxic chemicals
II, B2	ALL is EXHAUSTED OUTSIDE	Contaminated air is under/surrounded by NEGATIVE PRESSURE; outside exhaust must be hard-ducted	100 lfpm	Use with biologicals & SMALL amounts of volatile or toxic chemicals

Labconco "C1" BSC

- The Labconco Purifier Axiom Class II, Type C1 biosafety cabinet has recently been introduced to the market. The "C1" designation was generated by the manufacturer and is not currently recognized in the industry by NSF, NIH or CDC although it is designed to protect you, the product and the environment, as with other BSCs.
- This cabinet is marketed to be more flexible than previously available biosafety cabinets because it can be used as a re-circulating Class II Type A cabinet for standard microbiological work, or it can be ducted to the outside (or used with a manifold exhaust system) for working with volatile chemicals and radionuclides, as with a Type B cabinet.
- Special HVAC requirements may be involved with the installation of this type of cabinet and all manufacturer's recommendations must be followed.

4.16.3 Using Volatile Chemicals/ Radionuclides in a BSC

- In general,
  - Minute amounts can be handled in Class II Type A2 or B1 BSCs vented to outside.
  - Small amounts can be handled in Class II Type B2 BSCs vented to outside.
- Specifically,
  - Check the Safety Data Sheets for volatile chemicals to learn explosion limits, and avoid approaching those concentrations, as chemicals can volatilize and concentrate to hazardous levels in a BSC or after being pulled through HEPA filters.
  - Make sure the volatile chemicals you use will not damage HEPA filters.
- You have exceeded the quantity of a volatile chemical you can safely use in your BSC if you can smell or otherwise detect chemical fumes out in the lab. In these circumstances, discontinue use of that BSC; instead, locate and use a BSC that exhausts most or all air to the outside.

4.16.4 BSC Certification and Decontamination Requirements

- All new BSCs to be used for handling potentially infectious/biohazardous material must be certified before being used; certification involves a standardized check of proper function, performed by a qualified technician.
- Yearly certification is required for any BSC which is being used with potentially infectious materials.
- Cabinets must be re-certified 1) following a move to a different location; 2) if a HEPA filter is replaced; 3) following any other repair or service to the unit.
- Prior to the technician's arrival, prepare a BSC to be certified by removing all items and disinfecting all work area surfaces.

- Work surface decontamination is usually sufficient for certification, in situ repairs and moves within a building.

- Decontamination of the entire unit by a qualified contractor is required prior to a major relocation, going to surplus, receiving extensive repairs, or following a high-volume spill.

- Disinfecting agents typically used by professionals include formaldehyde gas and vaporized hydrogen peroxide.

- If you need assistance in determining whether whole-unit decontamination is needed, or for information on scheduling decontamination service with a contractor, contact EHS.

4.16.5 Guidelines for BSC Use

- Cabinet blowers should be operated at least 5 minutes before beginning work to allow the cabinet to purge any unfiltered room air within it. This purge will replace the atmosphere in the cabinet with filtered air.

- The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window glass should be wiped with a solution of 70% ethanol or other appropriate disinfectant.

- The surfaces of all materials and containers placed into the cabinet must be wiped/sprayed with 70% ethanol or other appropriate disinfectant to reduce the introduction of contaminants into the cabinet environment.

- Place all necessary materials in the BSC **before** beginning work. This will serve to minimize disruptions across the air barrier of the cabinet.

- Disruption of the air curtain occurs with rapid movement of a worker's arms into and out of the cabinet, compromising containment provided by the BSC. Move your arms in and out slowly, perpendicular to the face opening of the cabinet, to reduce this risk.

- Other personnel activities in the room (e.g., walking back and forth behind someone working at a BSC, entering/exiting lab, opening/closing lab doors, etc.) may also disrupt the cabinet air barrier. For this reason, access to the work area must be restricted when work is in progress.

- Before beginning work, adjust stool height so that your face is positioned in front of the BSC glass and is well above the opening below the glass that accesses the BSC work surface.

- Manipulation of materials should be delayed for approximately 1 minute after placing the hands/arms inside the cabinet, which allows cabinet's air flow to stabilize.

- When the user's arms rest flatly across the front grille (partially blocking it), room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly

will alleviate this problem. The front grille must not be blocked with paper notes, discarded plastic wrappers, pipetting devices, etc., and the back grille must not be blocked by supplies, waste containers, pipette tip boxes, racks of tubes, etc.

- All operations should be performed on the work surface at least 4 inches from the inside edge of the front grille.
- Equipment that causes air turbulence (e.g., centrifuge, vortex, etc.) should be placed in the back 1/3 of the work surface. All other work in the cabinet should stop while equipment is running.
- **Do not use open flames such as Bunsen burners inside the BSC. Open flames create turbulence that disrupts the BSC protective air flow which could expose personnel to biohazardous material. In addition, if the burner is turned off incompletely, gas can accumulate in the BSC enclosure, creating a risk of explosion. Flame-sterilizing tools in a BSC using a burner and alcohol is very hazardous; if alcohol is ignited, the heat and flame will likely destroy a HEPA filter. Instead of an open flame, use a Bacti-Cinerator, glass bead sterilizer or disposable loops/spreaders.**
- Separate clean and contaminated items. Minimize movement of contaminated items over clean items (i.e., work from clean to dirty).
- Only the materials and equipment required for immediate work must be placed in the BSC. Do not use the BSC as a storage area.
- Prior to removal from the cabinet, used pipettes and other contaminated material must be decontaminated or placed into a sealed container which is disinfected on outside surfaces.
- All vacuum lines must have in-line HEPA filters and traps containing disinfectant; all vacuum filtering takes place in the biosafety cabinet.
- At the end of the work session, all materials and work surfaces must be decontaminated with appropriate disinfectant. The work surface, the interior walls, and the interior surface of the window are again wiped with 70% ethanol or other appropriate disinfectant. A UV light may be turned ON for 30 minutes as an additional precaution, with the understanding that UV radiation is effective upon surfaces only (does not penetrate) and is no substitute for decontaminating the work area with disinfectant. UV lights should be activated in a BSC when personnel are not in the immediate vicinity to avoid eye/skin exposure to UV radiation.

4.17 Aseptic Technique — Specific Tips

- *Maintaining purity of cultures by use of aseptic technique is essential to producing valid research results.*
- Work Area

- Culture space is in a separate room, or separated from other areas of activity in the lab.
- Access to culture space is limited or restricted during a work session.
- Culture area is kept as clean and uncluttered as possible.
- Air temperature in culture area is cool enough for lab workers wearing PPE to work without becoming overheated.
- Incubators, shakers, etc. are routinely cleaned and decontaminated.
- Certain incubators, BSCs, etc. are designated for use with specific culture types, i.e., a dedicated tissue culture BSC where no bacteria or fungal culture work is performed that would present a contamination risk.

- Containment

  - BSC blower velocity and directional air flow is qualitatively checked with every use.
  - At least 5 minutes for air purge is provided in the BSC in between work sessions with different cell lines, and consider waiting up to an hour between sessions with different organism types (e.g., bacteria/tissue cells/ fungi, etc.).
  - Open flames are not used in the BSC.
  - Vortex mixers are positioned in the back third of the BSC work surface.
  - BSC work surface is populated with needed supplies only for a work session.
  - Items/supplies on BSC work surface are laid out in a 'clean to dirty' arrangement, and the flow of work proceeds from the clean area to the dirty or contaminated area.
  - The BSC work surface is not overcrowded with items, and is arranged so that an open space is provided for culture work to take place.
  - Pipettes are discarded in a tray with disinfectant on the 'dirty side' of the BSC work surface and the tray is removed after the work session; pipettes should not be discarded outside of the BSC during the work session.
  - Each item is thoroughly spray-disinfected before placement in the BSC.
  - Each contaminated item is thoroughly spray-disinfected before removal from BSC.

- Reagents, Stock Solutions and Media

  - Each lab worker uses his/her own stock solutions and media rather than sharing them among lab workers.
  - Each cell line has stock solutions and media reserved for use only with that cell line.
  - Lab workers aliquot stock solutions/reagents ahead of time rather than making stocks during culture sessions.
  - Sterility of stock solutions are checked by culturing for no growth.

- PPE, Hygiene and Behavior

  - Hands, wrists and forearms are washed before donning PPE, and PPE is worn so that there is no exposed skin on forearms between glove and lab coat cuff.
  - Disposable gloves that are resistant to ethanol are used.

- Gloved hands are sprayed with 70% ethanol before and frequently during culture work.
- Clean, cuffed lab coats are worn.
- Seat height is adjusted so that the lab worker's face is fully above the opening to the work surface.
- Talking with coworkers is limited while culturing to better attend to the work.

- Work Surfaces

  - Surfaces are disinfected thoroughly and systematically, i.e, from one side to another, wiping over each section only once.
  - A liberal amount of disinfectant is used and left on surfaces for needed contact time.
  - Surfaces are disinfected before and after every separate culture session.
  - Spills are cleaned up thoroughly and immediately.

- Good Habits

  - Culture containers are labelled well.
  - Records for frozen cultures are well maintained.
  - A log for media/reagent lot numbers is kept.
  - An ample supply of frozen culture material is maintained.
  - Culture workloads are manageable for lab workers.
  - Lab workers strive to do one thing at a time, one culture at a time.
  - Lab workers are proficient in aseptic pipetting, uncapping/capping, and pouring techniques with culture material.
  - Lab workers avoid reaching over open or clean items during culture work.
  - Lab workers evaluate their work flow periodically, and makes adjustments as needed.
  - New lab workers are trained by a skilled worker before performing independent work at the bench.
  - Training is documented.
  - Proficiency checklists for training are utilized.
  - A designated person in the lab manages training of new lab workers.

- Detection and Management of Contamination

  - When a contaminated culture is found, it is isolated from clean cultures. It discarded and a new culture is started from frozen stock rather than trying to recover purity through aggressive antibiotic treatment.
  - Cell cultures are kept antibiotic-free as much as possible so that low-level contamination can be more readily detected.
  - Tissue cell cultures are examined microscopically every time they are handled; lab workers are familiar with the normal morphologies of cell lines used in the lab, and can recognize deviations caused by contamination or other abnormal conditions.

- Tissue cell lines are checked for bacterial/fungal contamination by culturing for these contaminants on a regular basis.
- **Purity and identity of all cultured material is verified; bacterial/fungal stocks are checked for pure culture on a regular basis, and/or before experimentation.**
- Cell cultures are checked for mycoplasma contamination at a regular interval.
- Vented, secure closures are used on culture flasks.
- Use safety caps/sealed rotors when centrifuging biohazardous material.

4.18 CO_2 Incubation

4.18.1 Overview

- Many cultured biological materials require an atmosphere of enhanced carbon dioxide in order to thrive, thus CO_2 incubators are common pieces of lab equipment in bioresearch. Successful use of these incubators requires training, knowledge, and a dedication to cleaning and maintenance; the following procedures are provided to aid in this effort.

- Due to the supply of compressed gas needed, EHS online *Compressed Gas Cylinders* training is required for users of these incubators.

- Hazards:
  - CO_2 compressed gas cylinders present:
    - An explosion hazard, as does any compressed gas
    - A physical hazard, i.e., potential for injury if improperly securing, transporting or manipulating a heavy tank
    - An asphyxiation hazard if carbon dioxide rapidly escapes and replaces oxygen in an enclosed space
  - Stackable incubator units can cause a crush injury if not properly secured, as well as strain injuries upon lifting to stack units. Make sure that the water jacket is empty when stacking a unit to lessen the weight to be lifted.
  - An exposure potential exists from incubation of biohazardous materials; wear PPE when handling cultured material, cleaning up incubator spills, and for routine cleaning.

4.18.2 Positioning a CO_2 Incubator

- Avoid placing a CO2 incubator directly on the floor. This is because dust/dirt can be swept directly into the chamber with each door opening. Use a support stand to raise up the unit from floor level.

- CO2 incubators can be stacked, but only stack similar brands. Stacking dissimilar units risks an accident because there may be no way to secure the upper unit to the lower one.
- When using stacked units, never open both exterior doors at the same time; this imbalances both.
- Make sure there is a 3-inch clearance on all sides of the unit for ventilation of gas, for heat release and for access to power cords and connectors.
- Never lift a CO2 incubator using its door; lift using bottom side edges only.
- Place the incubator away from traffic areas, and from ventilation or other air streams.
- Do not place an incubator in direct sunlight, which could affect internal temperatures.
- Avoid placing in damp/humid and unventilated corners, which could increase the potential for fungal contamination.
- Consider the potential for vibration from nearby equipment or hallway traffic, which can affect cell growth. Locate incubators away from vibration sources, or use an anti-vibration mat.

4.18.3 Setting Up a CO_2 Incubator

- Make sure gas hoses are tightly sealed onto the connecting filter, and secure them with hose clamps.
- Clean the interior walls of the incubator, shelf supports and shelves before using it.
- Fill the humidity pan with sterile distilled water ONLY; water should have a resistance range of 50 Kiloohms to 1 Megaohm. Reason: *Tap wate*r with chlorine can corrode stainless steel, and will contain contaminants and minerals. *Deionized or ultrapure water* is very corrosive for stainless steel due to low ionic content, and tendency to absorb CO_2 which will react with air to form carbonic acid. *Reverse osmosis (RO) wa*ter can vary tremendously and thus is not recommended.
- A two-stage CO2 pressure regulator on the outlet valve of the CO_2 cylinder is required; a single stage regulator will not maintain the 15 +/- PSIG needed for proper operation.
- Before using electrical equipment to use inside a CO_2 incubator, test how much heat will be generated by the equipment within the incubator. Incubators cannot always compensate for additional heat loads. NOTE: Shakers create both heat and vibration; if an incubator unit can handle the heat load, a shaker should be run at < 150 RPM to keep vibration down.

4.18.4 General Maintenance

- Replace HEPA filter and gas inlet filters **every 6 months to one year**.
- Clean the fan and fan wheel **2-3 times a year**.

- Replace the water in the humidity pan **weekly or biweekly**---don't just refill. DO NOT let the water in the pan completely evaporate! This will cause water to evaporate out of cell culture media, resulting in higher salt concentrations and cell death. Low humidity in a CO_2 incubator can also damage internal sensors. NOTE: Regarding antibacterial options to use in the water pan, use only products that are recommended specifically for CO_2 incubator humidification pans/reservoirs.
- Check the internal temperature of a CO_2 incubator **every 3 months** using a National Institute of Standards and Technology (NIST) thermometer.
- Check the CO_2 level **every month** using a fyrite gas analyzer, or an external IR (infrared) tester.
- Keep a maintenance log for your CO_2 incubator, and record when these maintenance tasks are completed.
- The cleanliness of the entire lab is important to prevent airborne dust/dirt from finding its way inside the CO_2 incubator. Remove cardboard boxes from lab (which generate dust), clean the lab every few weeks and use sticky mats to reduce dust and dirt from foot traffic. Pay special attention to cleaning these areas:
  - Corners of rooms
  - Tops of equipment, under equipment
  - Water baths
  - Centrifuges
  - Microscopes
- Use 70% ethanol to disinfect the incubator interior. NEVER use chlorine-containing cleaners, as it is corrosive for stainless steel and bleach fumes are toxic to cells. NOTE: Incubators with copper interiors are recommended (but are expensive); 100% copper prevents contamination, is easy to maintain, and is safe for cells.

4.18.5 Best Procedure for Manual Cleaning of a CO_2 Incubator

- Cleaning frequency: every 1-2 weeks, or at least monthly.
- Wear lab coat and gloves.
- Move all cultures to a different incubator, or store in clean, disinfected plastic box if cultures will not be harmed by sitting at room temperature.
- Turn off incubator and gas supply.
- Remove:
  - shelves, supports and brackets
  - sensors as needed
  - water pan/reservoir (and empty)

- Wash all the parts removed with mild soap and warm water, and rinse with distilled water. Wipe dry with a clean, lint-free cloth. At 6-month intervals, autoclave cleaned shelves, supports/brackets and water pan.
- Clean internal surfaces, inner glass door and door gasket with mild soap and warm water. Be sure to reach all corners and crevices. Rinse with distilled water and wipe dry with a clean, lint-free cloth.
- Spray the interior of the incubator with 70% ethanol; do not spray sensors. Spray shelves, supports/brackets, and water pan with 70% ethanol and replace them in the incubator. Wipe door gasket with 70% alcohol. Shut door and turn on incubator to reach a warm temperature, and let interior air-dry completely.
- Fill water pan with sterile distilled water.
- Turn on the CO_2 gas and allow level to equilibrate.
- Using a lint-free cloth dampened with mild soapy water, wipe the exterior surfaces of the incubator (paying special attention to door handles), then wipe again using a clean cloth dampened in clear water. Avoid spraying ethanol on touch-sensitive areas, like touch-control panel; these and door handles can be wiped with an alcohol-moistened cloth.
- Return cultures to chamber when incubator has reached set conditions.

4.19 PipettIng And Pipette Disposal

4.19.1 Use

- Pipetting is an essential tool in laboratories to transfer precise amounts of fluid through the aspiration and dispensing actions of adjustable-volume, mechanical pipettors. Factors affecting pipettor performance:
  - How well users are trained
  - User fatigue
  - Air temperature, relative humidity and temperature of samples
  - Integrity of the mechanical components
  - Corrosive effects of fluids
  - Frequency of calibration
- ***Never mouth-pipette with serological pipettes*.**
- Pipettors utilized in the BSC must be properly identified for use with biohazardous materials with a biohazard label. They must be sprayed within the cabinet with appropriate disinfectant before work begins, and after work is finished.
- Use disposable plastic serological pipettes when possible.

- Pipetting procedures will produce aerosols and must be conducted in a BSC. Avoid these activities:
    - Mixing biohazardous fluids by repeated suction and expulsion from pipettes.
    - Bubbling air through biohazardous fluids.
    - Forcibly expelling liquids from pipettes.
    - Dripping infectious liquids from pipettes.

- To reduce aerosols, discharge fluid down the side of a container and avoid touching the pipette tip to the container.

4.19.2 Pipettor Calibration

- Pipettor calibration is necessary for meaningful research to take place. How often you should have laboratory pipettors calibrated depends on:
    - The skill and care with which they are used
    - How intensively they are used
    - The type of liquid being dispensed; volatile or corrosive liquids may emit vapors or contact metal parts, affecting performance
    - The type of disposable tip being used--whether or not you are using tips recommended by the pipette manufacturer
    - The level of accuracy and precision required; greater accuracy demands more frequent calibration

- Under normal conditions in a research lab, most pipettors can be calibrated every six months. The FDA or Good Lab Practice (GLP) requirements may specify quarterly calibration, and very critical applications may require monthly calibration.

- The best way to keep lab pipettors performing accurately is to have them professionally calibrated. Some calibration technicians will come to you and perform the work in your building or lab; other calibration companies require you to ship the pipettors to their facility for service, afterwhich they are shipped back to your lab.

- Depending on user requirements, most pipettor calibration companies can provide a range of service, from basic certification to detailed statistical analyses of pipette performance.

- Pipettor calibration can be accomplished in-house, but requires specific equipment and expertise.

4.19.3 Disposal

- Discard Pasteur pipettes and pipette tips into bio-SHARPS containers.
- Discard serological pipettes into a disposable pipette box, a lidded tray with disinfectant, or a small Biohazard autoclave bag located inside cabinet.
- Any of these containers must be closed and outer surfaces sprayed with disinfectant before removal from BSC.

- Aspirating 5% bleach solution through a pipette before discarding is recommended.
- It is important for disposable pipettes to be discarded into appropriate receptacles inside biosafety cabinets to avoid repeatedly breaking the air barrier of the cabinet by discarding in a receptacle outside the cabinet.
- Pipette receptacles must be closed when full and/or at the end of a work session. Autoclave in a timely way to decontaminate, then discard in Regulated Medical Waste.

4.20 Centrifugation

4.20.1 Overview

- Centrifuges are used in many aspects of laboratory research to separate or concentrate particles in a liquid medium. Tubes/bottles of samples are placed in balanced positions in rotors (the mobile part of a centrifuge); when loaded rotors are rapidly spun by a centrifuge, constituents in the samples will sediment differently according to their physical properties, and the sample's density and viscosity. Some centrifuges are refrigerated to reduce the frictional heat created by this process.

- Centrifuge types:
  - Microfuge or microcentrifuge – Small benchtop centrifuge that accommodates small tubes with capacities of 250 microliters to 2.0 milliliters; spins up to 15,000 rpm.
  - Benchtop – Counter-top models typically spin from 10,000 to 20,000 rpm.
  - High speed centrifuge -- Usually floor model; spins at 26,000 to 30,000 rpm.
  - Ultracentrifuge – Usually floor model; spins up to 100,000 – 150,000 rpm.

- Rotors for centrifuges typically are made of dense, heavy material to create momentum when spinning, and thus require less energy input to keep spinning. Rotors are often stored in refrigeration to keep them at or near centrifuge refrigeration temperatures.

- Different makes and models of centrifuges use different rotors, and each model comes with a table or graph that relates centrifugal force to rotational speed (rpm) for each rotor or swing bucket it can use.
  - DO NOT EXCEED THE PARAMETERS ON THESE TABLES/GRAPHS WHEN SETTING CENTRIFUGATION CONDITIONS.
  - ONLY USE THE SPECIFIC ROTORS AND BUCKETS LISTED ON THESE TABLES/GRAPHS WITH A PARTICULAR CENTRIFUGE; using a rotor that is not designed for a centrifuge is hazardous. It also could ruin both rotor and centrifuge.
  - A given centrifuge typically offers several rotor or bucket types/sizes for flexibility in choosing centrifugation conditions, and accommodating various sample containers.

- The centrifugal force (expressed in number of gravities, or # *xg*) generated is proportional to the rotation rate of the rotor (rpm) and the distance between the rotor center and the sample tube. *In lab write-ups, always record the centrifugal force used (# of gravities), and the duration of time*

elapsed while that force was applied; this is because centrifugal force is the only transferable unit among different centrifuges.

4.20.2 Centrifuge Hazards

- Physical hazards --
    - Injury or death can occur if centrifuge mechanical failure leads to loss of integrity and explosive breakup during operation. Damage leading to failures can be caused by 1) metal stress or fatigue reaching a critical state, 2) rotor corrosion or structural damage (i.e., forced loading of rotor onto a centrifuge spindle such that the alignment fittings are disabled and rotor is unmoored), 3) grossly imbalanced sample load in rotor, 4) attempted use of a rotor not designed for the unit.
    - Back injury can occur from leaning over to lift heavy rotors.
    - Crush injuries can occur from dropping a heavy rotor on hands/feet.
    - PREVENTIVE MEASURES:
        - Ensure that centrifuges and rotors receive regular preventive maintenance from a qualified service provider.
        - DO NOT USE A DAMAGED ROTOR; report it to a supervisor.
        - When operational problems appear (vibration, etc.), take centrifuges out of service immediately until repairs can be made.
        - Keep rotors clean and free of sample residues to prevent corrosion; corrosive damage can progress over time and eventually cause structural failure. Have your service provider monitor rotors for this damage on a regular basis.
        - Pay attention to your posture and plan your actions when lifting, carrying or placing heavy rotors.

- Exposure hazards due to aerosolization of biohazardous materials --
    - Centrifuges can be used with biohazardous agents only if they are equipped with solid covers and have safety interlocks that prevent opening until the centrifuge has come to a complete stop.
    - Per their risk assessments, some biohazardous materials require that respiratory protection is available in case of a spill when centrifuging. If you are uncertain, check the agent's SDS or risk assessment, or consult with EHS to determine if respiratory protection is required for spill situations.

4.20.3 General Guidelines for Centrifuging Biohazardous Material

- Use a centrifuge only after you have been trained by an experienced person on how to do it safely; it is recommended that you read applicable parts of the unit's operations manual as well.
- Make sure that your sample containers are rated for their intended use, i.e., for the speed, temperature and chemical resistance needed.
- Wearing appropriate PPE, fill sample containers no more than ¾ full in the BSC.

- Securely close sample containers (tubes, bottles, etc.) for centrifuging.
- Always check buckets and rotors for cracks, deformities, wear or other damage, and for proper gasket/O-ring condition and placement prior to use.
- Use sealable rotors or buckets with safety cups whenever possible as an added containment barrier in case a sample container leaks during centrifugation.
- Wipe or spray exterior surfaces of sample containers with disinfectant before loading into rotor in the BSC.
- Carefully balance the sample containers in the rotor. Use a weight scale to balance pairs of tubes/bottles if necessary.
- Wipe or spray-disinfect exterior surfaces of loaded rotors/buckets before removing from BSC.
- Stop a centrifuge immediately if it begins to operate in an atypical way during your run (vibration, unusual noise).
- Wait at least 10 minutes after the spin has stopped before opening the centrifuge lid.
- Don appropriate PPE and open the centrifuge; check to see if there has been a release of sample material. If all is well, put on appropriate PPE, remove rotor to BSC, remove samples, and clean any residue left in rotor by sample containers; leave rotor clean.
- Decontaminate bucket/rotor surfaces and centrifuge interior surfaces after use with biohazards and return rotor/bucket to its storage area.

- After opening the centrifuge, if you see a release of liquid outside of the sealed rotor or bucket (or from primary tube/container if not using a sealed bucket or rotor), close the centrifuge immediately, inform coworkers of the spill, place a "Stay Away—Spill" sign on the unit, and wait at least 30 minutes before initiating clean up to allow aerosols to settle. Then follow **1.8.7 Spill Occurring Inside Centrifuge**

4.21 Flow Cytometry

- Flow cytometers are technically sophisticated instruments that measure quantitative properties of single cells, such as:
  - Cell size
  - Total DNA
  - Newly synthesized DNA
  - Messenger RNA
  - Specific surface receptors
  - Intracellular proteins
  - Transient signaling events in living cells

- Measurements/counts are taken by passing a suspension of unclumped cells, in single file, through an electrified aperture causing changes in voltage, or across a laser beam where scattered light is measured. In some instances, the cell suspension is then discarded; in other situations, cells are actually sorted by this method into separate containers.

- Instrument Types
    - Benchtop analytical flow cytometers -- Utilized in an individual PI's lab, or in a core facility.
    - High-speed cell sorter flow cytometers – Typically utilized in a core facility; this type of instrument aerosolizes samples via a nozzle that forms a jet of micro-droplets, and thus cannot be used for sorting infectious or potentially infectious samples unless contained in a BSC, or by another approved containment method. Fixed materials may be sorted outside of containment.

- Laser Hazards
    - In most instances, flow cytometers contain safety devices that prevent user exposure to laser beams. Safety covers would have to be intentionally removed, or interlocks intentionally inactivated for laser exposure to be hazardous.
    - Users must be trained to avoid staring at the objective/ specimen while the instrument is scanning.

- Biohazards
    - Instrument malfunctions such as a clogged nozzle or air in the fluidic system can drastically increase aerosol formation, thus routine maintenance and preventive maintenance on equipment is essential for laboratory safety.
    - Exposures can occur from sample handling as well as from aerosols. Best practice: Handle all live samples for submitted for cell cytometry as biohazardous material.
    - Cell sorter operators who handle and process specimens without being aware of all details concerning the nature of the specimens are placed at increased risk. Thus, a completed questionnaire should be submitted with samples for flow cytometry which would supply the operator with information about sample origins, whether they are live or fixed, and if chemicals, nanoparticles, potential pathogens or genetically modified material are present in samples. **It is incumbent upon the person requesting cell analysis/ sorting to thoroughly divulge information on all potentially hazardous materials (biological or chemical) in specimens submitted.**
    - Samples that must be treated as potentially biohazardous for these procedures are:
        - Samples containing recombinant nucleic acid material (e.g., rDNA, rRNA, viral vectors, transgenes, etc.)
        - Samples containing lentivirus, adenovirus, or any genetically engineered amphotropic virus

- Unfixed human or NHP specimens
- Unfixed cells from primary or immortalized cultures of human/ NHP origin
- Unfixed cells from primary and immortalized cultures from animal donors that could be potentially infectious with zoonotic agents, and from transgenic animals
- CD34 stem cells, tumor cells, transfected tumor cells
- Samples containing nanoparticles

- Risk Assessment for Cell Cytometry
  - A laboratory conducting flow cytometry must identify its specific biohazardous risks from every angle -- specimen processing/handling, aerosol containment, waste management, equipment maintenance, operator training level/ proficiency/ experience, and PPE – and develop mitigating practices and procedures for each. The following chart provides general guidelines per BSL for some sample types and agents.

Biosafety Level Guidelines for Cell Sorting (2014 ISAC Standards) http://onlinelibrary.wiley.com/doi/10.1002/cyto.a.22454/full

	BSL2	BSL-2 with enhanced precautions (during sorting operations)	BSL3	BSL4
Risk Assessment Condition	Uninfected non-primate cells	Non-infectious Human/NHP cells; Infectious but with low risk assessment	Infectious samples with high risk assessment; All samples containing known aerosol pathogens	Extremely Dangerous Pathogens
Example sample type or agents [a]	Normal murine cells third-generation Lentivirus (non-human cells)	Normal human blood; Human cell lines [a]; An example agent is: Influenza A [a]; second-generation Lentivirus or third-generation in human cells	Example agents include [a]: Mycobacterium Tuberculosis, Monkeypox	Example agents include [a]: Ebola, Marburg
Containment System Validated	Periodically (monthly or with filter change) [b]	Periodically (monthly or with filter change) [b]	Weekly or before Every Sort [b]	Weekly or before Every Sort [b]
Aerosol Containment Operational	Required	Required	Required	Required
Respirator	Optional	N-95, FFP2 or better [c]	PAPR	Special Suit
Eye protection	Safety Glasses	Face shield or safety goggles	N/A	N/A
Lab Coat	Front Closure lab coat	Wrap around, solid-front	Coveralls	Special suit
Separate Room and Environmental controls	Optional	Required or limited access to room [d]	Required [e]	Required [e]

a Example Sample type or Agents—the samples and/or agents listed represent only a partial list of agents, which may be included in each category. A risk assessment should be conducted for all samples/agents before sorting, and the appropriate biosafety level determined in collaboration with safety specialists, cell sorter operators, subject matter experts and the Institution's IBC or equivalent. For additional information please consult the following web sites: http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php; http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

b Frequency of testing will be dependent upon the risk assessment and consultation with biosafety professionals and/or the IBC or equivalent. For more detail see Section 3.1.1.1.

c Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened. Note that respirator protection may otherwise be removed during the sorting process providing the aerosol management system is active and all sort chamber and collection chamber doors are closed. For human pathogens, i.e. Risk Group 2 agents, which are classified as BSL2 and are not respiratory hazards, but which may pose a risk if exposed to mucous membranes, only mucous membrane protection is required. Examples of agents in this category include Leishmania and toxoplasmosis in murine cells.

d Enclosure of the cell sorter within a certified (see Section 3.1.1.2) Class II BSC may abrogate the need to house the sorter in a separate room within the BSL2 lab space; PPE (as detailed above) is optional, but strongly encouraged for the operator during procedures requiring manipulation of instrument. Cell sorters located within a shared laboratory may be operated under BSL2 with enhanced precautions if during the operation of the sorter, access to the room is limited and PPE as detailed above is worn by all occupants.

e Enclosure of cell sorter within a certified (see Section 3.1.1.2) Class II BSC required.

- Requirements for Flow Cytometry Using Biohazardous Materials (BSL-2)
    - Lab facilities must demonstrate negative air pressure, and possess required design and equipment features of a BSL-2 lab.
    - The lab must operate with closed doors and restricted entry (with appropriate signage posted) when potentially biohazardous materials are being analyzed or sorted.
    - A BSC must be used for biohazardous sample handling.
    - Aerosol containment must be achieved when biohazardous materials are analyzed/sorted, whether that is provided by BSC containment for equipment, by use of a portable aerosol management system (provides a negative pressure environment for a cell sorter by drawing air from the unit through a HEPA filter, then releasing the air into the environment) or by another method.

- Aerosol control measures on instruments must be tested periodically, and test results documented. Laboratories must develop and implement such testing methods (e.g., "Glo Germ"™ is a rapid and inexpensive product that can be used to provide good qualitative aerosol containment data.)

- Operators must wear appropriate PPE when handling and analyzing samples; at a minimum, this would include disposable gloves, lab coat or disposable gown, and eye protection.

- Work with certain materials may also require respiratory protection in the form of N95 respirators or PAPRs in addition to using a BSC for containment.

- Personnel Requirements
  - Personnel who operate flow cytometry equipment must be well trained on the instrument, as well as on safe handling of specimens, and use of safety equipment. They must also demonstrate a sound biological understanding of the types of materials handled/ processed, and the hazards, exposure potentials, routes of transmission, etc. associated with each.

  - Only fully trained personnel must be allowed to use this equipment.

  - Personnel who operate this equipment and may be exposed to biohazardous material must complete a Medical Questionnaire, and update it yearly.

- Waste Management
  - Effluent must be collected from the instrument into containers with fresh full-strength bleach or other suitable disinfectant, in sufficient quantity to achieve a 10% final concentration.

  - Fluid lines must be decontaminated routinely with freshly diluted (1% - 10%) bleach or other suitable disinfectant.

4.22 Other Aerosol-Generating Lab Equipment and Tasks

- *Lab equipment* that breaks up, slices, mixes, separates, nebulizes or applies fluidics to move biological material has the potential to create biohazardous aerosols, such as:

  - Homogenizers, blenders, sonicators, tissue grinders, microtomes, lyophilizers, nebulizers, lasers, shaking incubators
  - Centrifuges, vortex mixers
  - Fermenters, flow cytometers, pipettes

- *Research tasks* that have the potential to create aerosols include, but are not limited to:
    - Animal inoculations
    - Removing fluid from a vial with rubber septum with a needle
    - Harvesting egg or animal tissue
    - Necropsies
    - Opening a vial of lyophilized biological material
    - Opening a vial of thawed culture material
    - Flaming inoculating loops with open flame
    - Performing bacterial staining
    - Performing microscopy using live agents
    - Loading a hemocytometer for cell counting
    - Changing animal bedding

- Measures to reduce aerosol creation:
    - Think through each procedural step to identify the specific risks.
    - Eliminate, substitute or reconfigure, if possible, to lessen the aerosol risks.
    - Utilize all containment elements that are part of the equipment, i.e., safety cups on centrifuge rotors.
    - Utilize biosafety cabinets or other containment devices to house aerosol-generating small equipment or tasks.
    - Always disinfect work areas and equipment after use.
    - Always use appropriate PPE to protect yourself.

4.23 Toxins of Biological Origin (Biotoxins) – Handling and Management

- Biotoxins may cause death or severe incapacitation at relatively low exposure levels. They are produced by living organisms, but they do not self-replicate. Predominantly they are not man-made but are of natural origin; however, biological toxins are increasingly being synthesized by modern methods. They differ from chemical toxins in that they do not pose a vapor hazard and only a few (e.g., mycotoxins) are dermally active.

- Typical research applications of biological toxins include: use as a growth factor in cell culture media (e.g., cholera toxin), to produce specific neurologic effects (e.g., tetrodotoxin), and to produce localized tissue destruction (e.g., diphtheria toxin).

- Possession and research use of biological toxins or venoms requires:

  - Registration with, and approval by the VT IBC for research use of the toxin
  - Maintaining a toxin inventory record to account for current quantities in house, and toxin use and disposition
  - Storage of toxins in sealed, labeled containers within a secured (locked) storage device that can only be accessed by personnel authorized to work with the toxin
  - Use of toxins only in designated rooms with posted signage to control/limit access

4.23.1 Biotoxins as Select Agents and the Due Diligence Provision

- Some biotoxins are classified by the federal government as Select Agents due to their potential to pose a severe threat to public health and safety. Possession, use and transfer of these toxins is highly regulated.
- In small quantities, some of these agents are exempt from Select Agent registration but must still be registered with, and approved for use by the VT IBC .

Toxin	**Exempted Amount (≤)**
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Short, paralytic alpha conotoxins (containing the amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)	100mg
Diacetoxyscirpenol (DAS)	1000mg
Ricin	100mg
Saxitoxin	100mg
Staphylococcal enterotoxins (Subtypes A, B, C, D, E)	5 mg
T-2 toxin	1000mg
Tetrodotoxin	100mg

- Because it would be possible to stockpile toxins in multiples of these amounts which are small enough to be excluded from regulation, a 'Due Diligence' provision was developed in 2014, and places the following responsibilities on Principal Investigators, veterinarians or medical doctors:

IF YOU <u>POSSESS</u> ANY OF THESE TOXINS, YOU MUST:

1. Declare your possession and provide inventory documentation to Virginia Tech's Responsible Official (RO) and Alternate Responsible Official (ARO).
2. Contact the RO and/or ARO to schedule a SHORT informational session regarding requirements related to possession of these toxins.
3. If no additional toxin is received after the declaration date, but at least annually, you must verify and declare your possession to Virginia Tech's RO and ARO.
4. **Immediately** notify Virginia Tech's RO and ARO if your toxin inventory amounts, per toxin, ever exceed specified permissible limits.

IF YOU <u>TRANSFER</u> ANY OF THESE TOXINS TO ANOTHER ENTITY, YOU MUST:

1. Apply due diligence in assessing/ensuring that the recipient of these toxins has a legitimate need to handle/use such toxins.
2. Complete documentation **prior to the transfer** which records your information and the recipient's intended use of the toxin. The *Notification of Toxin Transfer* form (template can be found in the Appendices of this manual) must be submitted to Virginia Tech's RO and ARO.
3. **Immediately** report to Virginia Tech's RO and ARO, if a violation of federal law is detected, or if suspicious activity related to the toxin is discerned.

IF YOU <u>RECEIVE</u> ANY OF THESE TOXINS FROM ANOTHER ENTITY, YOU MUST:

1. **Immediately** notify Virginia Tech's RO and ARO of the receipt of the toxin, update your inventory, and submit inventory documentation to the RO and ARO.

CONTACTS:

Title	Name	Phone	Email	Mail Code
RO	Charlotte Waggoner	540-231-5864	ren@vt.edu	0423
ARO	Anna Kroner	540-231-1122	akroner@vt.edu	0423

4.23.2 Biotoxin Risk Assessment

- Biotoxins are assigned Biosafety Levels (which prescribes type of containment, PPE, work practices and safety equipment to use) by the same risk assessment process as biological agents.
    - Main Occupational Risks in the Lab
        - Accidental exposure by direct contamination of mouth, eyes or other mucous membranes
        - Inadvertent aerosol generation, such as when reconstituting a toxin in dry powder form
        - Needlesticks or other accidents that compromise the skin, such as could happen when inoculating animals
    - Risk assessments on biotoxins should include:
        - Biotoxin characteristics (LD_{50} in solution and dry form; solubility).

  - Risks inherent to experimental procedures and manipulations (e.g., opportunities for accidental needlesticks, the likelihood of dispersal from static build-up when working with powder form, etc.).
  - Total amount of toxin used relative to the estimated human lethal or cytotoxic dose.
  - Volume of material manipulated.
  - Availability of successful treatment, vaccines or antitoxins.
  - Training and experience of personnel.

- If toxins/ infectious agents/ animals are used in combination, then risks in all of those areas must be considered collectively in the selection of containment equipment and the development of safety procedures.

- Lab workers are permitted to handle toxins when they have 1) demonstrated proficiency in the pertinent lab procedures and animal handling techniques, if applicable; 2) completed all required general training and lab-specific training on toxin use with the PI or designee; and 3) completed and submitted the VT Medical Survey to the VT Occupational Health Program.

- Complex operations should be rehearsed without the use of live toxin in supervised practice runs until proficiency is assured.

- Safety measures should be selected according to a risk assessment for each manipulation involving the toxin. For example, if there is a skin-absorption risk in working with a toxin, gloves/sleeves must be used that are impervious to the toxin and the diluents/solvents used; if there's a splash or droplet hazard, safety glasses/goggles and face shield must be used.

- After risk assessment review, selected operations with toxins may require modified BSL-3 procedures; typically, routine operations (e.g., toxin preparation/ animal procedures) with dilute toxin solutions can be conducted under BSL-2 conditions using 1) a certified BSC or chemical fume hood, and 2) appropriate PPE for the hazards involved.

4.23.3 Mitigating Biotoxin Hazards

- Inhalation Risks
  - When working with dried toxin, remove all items that are not necessary for your procedure from the biosafety cabinet or fume hood before handling the toxin to reduce the potential for contamination of item surfaces.
  - Respiratory protection may be needed if you must manipulate a dry toxin in an open vessel, even if in containment. Consult with EHS.
  - Primary containers of the toxin should be non-breakable if possible, and vials should be maintained in a closed secondary container that will not allow escape of the product even if dropped. The risk of release is greatly decreased by using a secondary container at all times.

- Sharps Risks—Accidental Injection or Cut

- Use only syringes with luer-lock or integrated needles.
- Use vial adapters whenever possible as a substitute for using needles to add diluent through septums on vials.
- When introducing a needle through a septum, assure that the vial is secured with a device that allows the non-dominant hand to be outside of the 'strike zone' of the needle. (Example: secure vial in a rack, or use a clamp to hold vial instead of holding it directly.)

- Personal Protective Equipment
  - Wrap-around disposable gowns with gathered cuffs are the best choice, and fluid-resistant gloves rated for protection against diluent should be used.
  - Double-gloving is strongly recommended if it does not hinder the worker's dexterity to the point of being unsafe.
  - There should be no unprotected, exposed skin on your body or extremities when undertaking this work. Arms, wrists and hands must be fully covered; long pants and whole shoes are required.
  - Safety glasses are strongly recommended, and must be washed after removal and storage.
  - Disposable PPE used for this work must be single-use only, and disposed of as hazardous lab waste.

4.23.4 Solubilizing and Using Biotoxins

- Commercial preparations of toxins typically are in lyophilized, powdered form in crimped vials, topped by a rubber stopper. Individual labs sometimes re-package, aliquot and store the powder in vials or microcentrifuge tubes for later reconstitution. Either way, powdered toxin has to be solubilized in the lab, and this presents an exposure risk to lab workers due to the possibility of the powder being dispersed into the air.

- **General Precautions**
  - Remove toxins from the fume hood or biosafety cabinet only after the exterior of the primary container has been decontaminated and placed in a clean secondary container.

  - Toxin solutions, especially concentrated stock solutions, must be transported in a leak/spill-proof secondary container.

  - After a work session with a toxin in a fume hood or BSC, the interior surfaces of the unit must be thoroughly decontaminated; until this can be done, a sign must be posted on the hood or cabinet saying that the unit is awaiting decontamination, meanwhile toxin may be present so do not use.

  - Pressurized tubes or other containers holding toxins must be opened under containment of a BSC or fume hood.

  - Operations that expose toxin solutions to vacuum or pressure (e.g., sterilizing a toxin solution by membrane filtration) must be done in a BSC or fume hood.

- Vacuum lines used with toxin must be protected by a HEPA filter to prevent entry of toxins into the vacuum system.
- Centrifugation of materials containing toxins must be performed using sealed, thick-walled tubes, preferably in safety centrifuge cups or sealed rotors. After centrifugation, the rotor assemblies must be opened in a biosafety cabinet to remove tubes.
- Work with dry toxin should be minimized or eliminated when possible.

- **Preparing Biotoxin Stock Solution from Powder in Crimped Vials**
  - Work in a chemical fume hood.
  - Wear buttoned lab coat or disposable gown, and use gloves that are resistant to the diluent you are using.
  - Add diluent though the rubber stopper using a luer-lock or one-piece needle and syringe.
  - If necessary, allow the pressure differential within the vial to dissipate by withdrawing the needle above the meniscus and allowing the syringe plunger to be displaced.
  - Once the liquid has been added to the vial and powder has been wetted, dispose of the needle and syringe. A needle should not be used for handling, dispensing or aliquoting of toxin-containing solution.

- **Preparing Biotoxin Stock Solution from Powder in Microcentrifuge Tubes**
  - These containers are not meant to be penetrated by a needle. Opening the container top may lead to powder dispersal due to pressure differential, or static electricity. Spraying a static guard on gloves is highly recommended.
  - Gloves should be worn that are resistant to the diluent. In addition to gown, double-gloves, safety glasses and face shield, respiratory protection may be required.
  - Open container slowly behind a plexiglass shield in a portion of the lab isolated for this purpose, with limited air flow. Do not open the container in the vigorous air flow of a hood or biosafety cabinet. Do not open the container completely, just far enough to add the diluent.
  - After diluent is added, mix or vortex under a chemical fume hood. Once powder is wetted completely, the tube can be opened carefully without concern about air dispersal. When possible, tubes should be centrifuged to remove liquid drops from caps/lids.

4.23.5 Toxin Inactivation and Disposal

- Depending on the toxin, toxin-contaminated materials and toxin waste solutions can be inactivated by incineration, extensive autoclaving, or by soaking in suitable decontamination solutions; there is no universal method of inactivation. Deactivating aqueous solutions of a toxin may not be effective for inactivating the same toxin in dry, powdered form. Inactivation procedures should not be considered completely effective unless validated using specific toxin bioassays. Always consult EHS when considering the most safe and effective method of deactivation and disposal.

- Solid waste items (e.g., gloves, waste vials, bench paper, etc.) must not be soaked in a liquid decontamination solution.

- If a toxin is known to be inactivated by autoclaving, solid waste should be autoclaved before transport for final treatment and disposal. Consult EHS regarding a proper disposal method.

- If toxin cannot be deactivated by autoclaving, collect as Regulated Medical Waste for Incineration Only, label the container as containing Toxin Waste, and request EHS waste pickup.

- Sharps contaminated with a toxin may be disposed of in bio-sharps containers; label as containing Toxin Waste.

- If liquid waste containing toxin cannot be inactivated by autoclaving or bleach treatment, then it must be collected as chemical hazardous waste. Be sure to label as Toxin Waste, and contact EHS for hazardous waste pickup.

4.23.6 Toxin Spill Response

- Just as there is no universal inactivation method for toxins, spill response procedures must be tailored to the toxin being used, as characterized in the toxin's risk assessment. In general, spill response should include:
    - Immediately notify others in the area of the spill.
    - Don appropriate PPE, including respiratory protection if required.
    - Isolate the area (area should already be restricted when toxin is in use).
    - Remove the breached container; if non-glass, place in autoclave bag inside secondary containment. Place broken glass in sharps container.
    - Treat, absorb and remove spill contamination by covering spill with paper towels saturated with a decontaminating solution. Allow contact for 20 minutes. Absorb and remove the contaminated towels using tongs or other tools to minimize direct contact. Place materials in an autoclave bag.
    - Decontaminate all impacted surfaces in the 'splash zone.' Wait for the prescribed contact time to elapse before removing the residue. Use care to limit contact with PPE contaminated surfaces when removing PPE. Place PPE in autoclave bag.
    - All bagged or contained contaminated material should be labeled Toxin Waste and secured until it can be autoclaved, or in Regulated Medical Waste for Incineration Only containers, until EHS can pick up the waste.

4.24 Dual-Use Research of Concern (DURC)

- If proposed research could potentially provide knowledge, information, products or technologies that, if misapplied, could pose a significant threat with broad consequences to public health and safety, to agricultural crops and other plants, to animals or the environment, or to national security, then it qualifies as DURC.

- The U.S. government has designated the following agents as potentially involving DURC:
    - Avian influenza virus
    - *Bacillus anthracis*
    - Botulinum neurotoxin
    - *Burkholderia mallei*
    - *Burkholderia pseudomallei*
    - Ebola virus
    - Foot-and-Mouth Disease virus
    - *Francisella tularensis*
    - Marburg virus
    - Reconstructed 1918 flu virus
    - Rinderpest virus
    - Toxin-producing *C. botulinum*
    - Variola major virus
    - Variola minor virus
    - *Yersinia pestis*

- The U.S. government has designated these experiments as potentially involving DURC:
    - Enhancing the harmful consequences, or altering the host range/ tropism of an agent or toxin
    - Disrupting immunity or effectiveness of immunization against an agent or toxin without justification
    - Conferring resistance to an agent or toxin to interventions, or facilitating its ability to evade detection
    - Increasing stability, transmissability, or ability to disseminate of an agent or toxin
    - Enhancing susceptibility of a host population to an agent or toxin
    - Generating or reconstituting an eradicated or extinct agent or toxin listed above

- **If your research involves the use of one or more of the agents and experiments listed, it may be considered DURC.** DURC Researchers must comply with the U.S. Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. The government's oversight of DURC aims to preserve the benefits of the life sciences while minimizing the risk of misuse of this research.

- WHAT HAPPENS IF YOUR RESEARCH INVOLVES DURC:

1. You must submit a research protocol to the Virginia Tech IBC for review.

2. The A.V.P. for the Office of Research Compliance, the IBC, and the Office of Export and Secure Research Compliance will meet with you to discuss the research.

3. A mitigation plan will be drafted by you and those listed above, in accordance with the U.S. Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. The draft plan will be submitted to NIH and/or your funding agency for review and approval.

4. You will be provided with information and guidance by those listed above throughout the planning and approval process.

5. Your research cannot be initiated until your research protocol & risk mitigation plan is approved.

5. TRAINING FOR PERSONNEL WORKING IN OR AROUND BIOLOGICAL RESEARCH

Training must take place:

- **So that people can work knowledgeably and safely**
- **To meet local, state and federal requirements**
- **To produce scientific research of the highest quality**

5.1 Training Documentation Requirements

- *For liability and regulatory compliance purposes, we must:*
    - Document all training (i.e., initial, refresher, lab-specific topics, awareness, or remedial training).
    - Document proficiency evaluations.

- **Training records are subject to examination during inspections/ audits by oversight and regulatory agencies, and especially during investigations following an incident or exposure. Being able to prove that appropriate training took place can greatly impact the outcome of an investigation in the wake of a lab accident, e.g., fines imposed, criminal prosecution, etc.**

- **Completed training records (electronic or printed) must be retained for the duration of each person's employment or activity period in the lab, and for at least 3 years after a lab worker's separation date.**
    - Printed copies of training records can be kept in the Lab-Specific Biosafety Manual, or filed in an easily accessible location in the laboratory.
    - Electronic copies of training records can be stored in the lab's SMS records, or another local electronic storage site that is easily accessible to PI, Lab Manager, and lab personnel.
    - PIs or other supervisory personnel in the lab who have been designated as administrators for the lab in the SMS system can access and track EHS training records for the entire lab group.

5.2 Who Needs To Be Trained, How, And Why

- **<u>New personnel who will work with biohazards must receive:</u>**
    - GENERAL training – initial, foundational training in lab safety, biosafety, autoclave use, etc., *available in online EHS modules.* The following training modules must be completed by each person who will be working with biohazardous materials <u>PRIOR to beginning their work at the bench</u>:

- *General Laboratory Safety* (EHS online module)
- *Biosafety For Research Labs* (EHS online module)
- *Introduction to Biological Safety Cabinets* (EHS online module)
- *Safe Autoclave Use* (EHS online module)
- *Laboratory Hazardous Waste* (EHS online module)
- *Personal Protective Equipment* (EHS online module)

- These trainings have a 3-year expiration, at which time they must be renewed. Certain other trainings, such as Bloodborne Pathogens and Respiratory Protection, have an OSHA-based annual renewal requirement.

- Persons who work with rDNA molecules or synthetic nucleic acids must complete training on NIH Guidelines for Research Involving Recombinant DNA molecules .

- Additional training may be required or recommended, according to the person's occupational tasks/risks in the lab, such as *Compressed Gas Cylinder Safety* and *Portable Fire Extinguisher* training. Additional determinations may be made by EHS, IBC and Occupational Health review, in collaboration with the PI. Example: *Respiratory Protection*.

- Successfully completed EHS online training is recorded electronically. Trainees can access their online training profiles at any time through the EHS Safety Management System; it will display a list of completed trainings and their expiration dates, registrations for training not yet taken, and expired training. A record of completed EHS trainings can be printed from the SMS, if needed. The SMS also allows PIs/lab managers to view/monitor the training records of their lab groups for tracking completions, expired training, etc.

- SPECIFIC training-- initial orientation and training that is specific to your lab practices, equipment, procedures, and research. This is what is meant by "**lab-specific**" training, and *is provided by PI or designee.*

  - Use the ***Biosafety Training Record for New Lab Personnel*** as a guide for this initial, lab-specific training provided by PI or designee, and as a record sheet to document that training.

- **Existing personnel who work with biohazards will or could need:**

  - LAB TOPICS training on new techniques, equipment, biological agents, etc. as they are added to your research program.

  - REFRESHER training as needed, or at a regular interval as a lab group (annually, for example).

    - Use ***Lab Topics Training Record*** to document lab topics training or refresher training. The specific topic or reason for a session is entered at the top of the page, and signatures of participants in the training session are recorded on the page.

- REMEDIAL training when mistakes, exposures, accidents/incidents or 'near misses' occur, followed by a proficiency evaluation.

- HIGHER-LEVEL training and proficiency evaluation, such as for those preparing for lab management roles.

  - Use the ***Biosafety Proficiency Checklist*** (5 pages) as a customize-able guide for training, and for documenting proficiency evaluations for remediation or for higher level skills.

- *The PI or designee is responsible for providing these trainings and evaluations.*

- **Lab personnel from other groups with whom you share lab space, or share common-use equipment/ facilities need:**

  - AWARENESS training on the hazardous biological materials manipulated in your lab space or used with common equipment/ in common facilities. Bioawareness training should include:

    - the identity of the biohazards used or present, and what is done with them
    - how to avoid exposure to the biohazards
    - how to recognize signs/symptoms of disease caused by your agents, and how to respond if signs/ symptoms occur
    - how to respond to and report an incident involving your biohazards

    - Use the ***Bioawareness Training Record*** as a guide for bioawarness training topics, and to record the training for individuals or groups. The record sheet consists of a topics page, and an optional signature page if you are training a group.

  - *The PI or designee is responsible for providing bioawareness training.*

- **Personnel who work in or around the lab but do NOT work with biohazards need:**

  - AWARENESS training in biohazards as described above.

  - Use the ***Bioawareness Training Record*** as a guide for training topics, and to record the training for individuals or groups. The record sheet consists of a topics page, and an optional signature page if you are training a group.

  - *The PI or designee is responsible for providing this training.*

5.3 Guidelines For Trainers/ Proficiency Evaluators To Use

- The training record sheets/ checklists themselves (links provided in this manual) can serve as guides or templates. In addition, these documents can be customized by adding additional lab-specific topics as needed. PIs/ designees can also design their own guidelines or templates.

5.4 Training Delivery

The actual means by which trainees receive their lab-specific training is up to the trainer's preference.

Example 1: Create a stand-alone session (via PowerPoint, etc.) by using the training topics on the appropriate record sheet as an outline, then supply specific details for/about your lab, per topic. This method works well for initial training for new personnel, and for awareness training.

Maintain copies of any PowerPoint trainings that you develop in the file folder/ electronic folder/ section in your Biosafety Manual where you keep personnel training records so that auditors/ inspectors can see the training you provided. Actual delivery of such PowerPoint training can take place in a number of ways; for example:

- trainer presents it in person in a slideshow
- trainees view it on computers on their own schedules
- trainees work through it in hard copy in a notebook

Example 2: Trainer meets with trainees to provide information per topic, using the training record sheet as a guide. PIs/ designees can deliver initial lab-specific training, refresher training and awareness training to individuals or groups in this way. Also, this is the most common scenario when a technical specialist, visiting scholar or other person outside the lab group provides a "Lab Topics" training, e.g., a demonstration of a new technique, product, or piece of lab equipment.

5.5 Proficiency Evaluations

When mastery of knowledge, skills and lab techniques need to be assessed for lab personnel, proficiency evaluations are used. The PI can determine the content of the evaluation, and/or the Biosafety Proficiency Checklist can guide an evaluation. The Checklist can be customized to include your specific laboratory proficiency topics.

Prior to being evaluated, lab personnel may need mentoring/coaching/ extra training at the bench; they may also need guidance in finding or accessing educational material to read or study.

Proficiency evaluations must take place through a person-to-person interview between the individual being evaluated, and the person who is evaluating him/her; evaluators must have the necessary expertise and experience in pertinent topic areas. Besides interviewing the person to evaluate his/her knowledge and understanding, the evaluator must also observe the person at work.

5.6 Personnel Biosafety Training Summary

People To Train	Type of Training Needed	Training Document To Use	What the Document Certifies	Who Provides Training
New lab personnel who will be working with biohazards	EHS online training	Training is documented electronically in EHS training database and can be accessed on an individual's EHS Training Profile.	• Trainee is credited with successful completion of module. • Trainee name, date of completion of training, and when training expires	EHS -- online training modules
	• Biosafety training specific to your lab's procedures, equipment & agents. • Safety orientation (e.g., evacuation routes, safety equipment, reporting procedures, etc.)	***Biosafety Training Record for New Lab Personnel*** **(Serves as lab-specific training template for topics to cover, training record sheet, and record of proficiency)**	• Trainee understands topic areas covered. • Trainee is generally proficient in lab procedures covered. • Trainee/trainer names, signatures & date of training	Principal Investigator or designee
Existing lab personnel working with biohazards	• Biosafety refresher or review • Specific training on new procedures, equipment, etc.	***Lab Topics Training Record*** **(Trainer fills in the lab topic covered; trainees/ participants sign on a signature page)**	• Topics covered • Trainee/trainer names & date of training	Principal Investigator or designee
	• Higher-level, comprehensive lab training • Remedial training in one or more areas	***Biosafety Proficiency Checklist*** **(Serves as template for topics to cover and proficiency evaluation; can be customized according to need)**	• Trainee understands all topic areas covered. • Trainee has demonstrated proficiency in specific lab skills & methods covered. • Trainee/trainer names, signatures & date of training	
Lab personnel from other groups with whom you share lab space, facilities or equipment **OR** **Personnel working in/around lab but NOT with biohazards**	Lab-specific training to provide awareness of biohazards present in lab, & appropriate response to exposures, signs of disease, lab incidents	***Bioawareness Training Record*** **(Serves as template for topics to cover and training record sheet; can be customized according to situation)**	• Trainee understands topic areas covered. • Trainee/trainer names, signatures & date of training	

6. OCCUPATIONAL HEALTH FOR LAB PERSONNEL

6.1 Medical Questionnaire

- All personnel working with biohazardous material must complete a Medical Questionnaire. The questionnaire must be updated at least annually, and whenever your exposure potential to hazardous material/ activities changes.

- The medical questionnaire asks for 1) information on the hazards in your workplace and 2) for general medical history, as would be requested of you in a doctor's office. Medical history will only be collected on paid individuals or volunteers. For others, only workplace hazard information will be collected.

- The medical history portion of the questionnaire is optional. However, in some circumstances (e.g., working with select agents) if you decline to complete the medical history portion, your overall health status cannot be assessed and documented, which would prohibit your access to certain lab settings and activities.

- The information you supply on the questionnaire resides in a database at a secure site. It enables the confidential assessment of your workplace risk by licensed occupational health professionals at EHS who review the hazard information. The Virginia Tech occupational health nurse or physician reviews medical information. Based on these reviews, additional services or measures may be recommended or required for your protection.

6.2 Range of Services

- Occupational Health services that might be recommended/required for your safety (available at no cost to employees and graduate students) include:
    - Preventive vaccines for particular exposure risks (if a vaccine exists for the agent)
    - Participation in the Virginia Tech Respiratory Protection Program, including annual pulmonary function testing, respirator selection and fit testing, and training.
    - A PPE evaluation by EHS.
    - A medical examination with the Occupational Health physician.
    - Consultation with the Occupational Health physician for:
        - A potential or known exposure
        - Assessment of symptoms of disease possibly caused by a workplace pathogen
        - Assessment of an existing health condition that could put you at significant risk at work
        - Assessment of your safety in the workplace if you experience a change in health status

6.3 Personal Health Status Monitoring and Response to Symptoms

- Personal health status may impact an individual's susceptibility to infection, or ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel (especially, but not limited to, women of child-bearing age, immunocompromised individuals, persons suffering from chronic inflammatory conditions, cancer patients, organ transplant recipients, patients undergoing chemotherapy, radiotherapy, or immunosuppressive therapy) will be provided with information regarding immunocompetency and conditions that may predispose them to infection. Individuals having these or other medical conditions are encouraged to self-identify to EHS or the institution's healthcare provider for appropriate confidential counseling and guidance.

- All personnel must monitor their daily health status for signs and symptoms of disease consistent with the agents being manipulated in their laboratories. It is highly recommended that personnel familiarize themselves with those signs and symptoms so they can more effectively self-monitor. Self-report any such symptoms to the PI and/or lab manager, and/ or to EHS without delay.

- A medical evaluation will be provided for individuals following an exposure incident to potentially infectious material.

- Occupational Health records are kept in by the EHS Occupation Health Office for duration of each person's employment and for at least 30 years after. Research laboratories must not maintain any medical records for personnel.

7. BLOODBORNE PATHOGENS INFORMATION

7.1 What Puts Lab Workers At Risk For BBP Exposure, Level Of Risk, And Exposure Controls

- **If you work with or around one or more of the biohazardous materials listed below in your laboratory research, you are considered at risk for BBP exposure:**
    - Human blood/ blood products/ blood components
    - Human or NHP tissue cell cultures (primary or established lines)
    - Unfixed tissue or organs from humans, living or dead
    - Other potentially infectious materials of human origin: sexual fluids, cerebrospinal fluid, organ fluids, joint fluids, amniotic fluid, saliva (from dental procedures), or any body fluid containing visible blood
    - HIV or HBV-containing cell or tissue cultures, culture medium or other solutions
    - Blood, organs, other tissues or cell lines from experimental animals infected with HIV or HBV

- **Practices that increase your exposure risk in the lab include:**
    - Use of needles with no safety devices
    - Using needles preferentially over alternative or safer means
    - Careless handling and disposal of sharps (e.g., needles, broken glass, scalpels, blades, etc.)
    - Failing to use splash guards, face shields, safety glasses, disposable gloves or lab coats in situations that require them for protection
    - Failing to use containment equipment
    - Failing to wash hands
    - Failing to properly decontaminate surfaces and lab equipment
    - Failing to properly decontaminate and dispose of hazardous waste
    - Failing to follow Universal Precautions

- **Your risk of infection resulting from an exposure depends on:**
    - The specific pathogen involved and its concentration in the contaminated material
    - The type and volume of contaminated material you contacted
    - The way you contacted the material, and for how long
    - How effectively you used PPE

- **The typical research lab worker who works responsibly with established human cell lines has a very low risk of BBP exposure. Lab workers at a higher level of risk include those who:**
    - Collect and /or manipulate human blood, tissue or fluid specimens.
    - Work with primary human cell lines which have not been evaluated for presence of pathogens.
    - Perform research experiments with HIV or HBV. In all risk situations, however, the consequences of an exposure leading to infection are immense, thus an abundance of caution must be practiced.

- **Exposure control practices to follow in the lab (See Table in Section 7.4):**
    - Use of safety needles
    - Minimizing use of needles/ sharps; proper handling and disposal of sharps
    - Use of PPE to protect hands and mucous membranes; use barriers and containment equipment to protect against aerosols, sprays, splashes, etc.
    - Washing hands every time after glove removal and before leaving lab
    - Following surface disinfection and lab equipment decontamination procedures
    - Properly handling of hazardous waste/sharps waste
    - Following Universal Precautions

7.2 What Is Considered A Bloodborne Pathogen?

"Bloodborne pathogen" is a phrase applied to a variety of bacteria and intracellular parasites that can infect humans via the blood stream and cause disease. Human Immunodeficiency virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are the BBP of greatest concern, but there are a number of other significant pathogens that can be transmitted from exposure to human blood, some of which are involved in research on this campus.

7.3 How The OSHA Bloodborne Pathogens Standard Applies To Lab Workers

- OSHA Bloodborne Pathogens Standard 29 CFR 1919.1030 prescribes safeguards to protect at-risk workers against the health hazards caused by BBP, and these safeguards must be provided by employers as mandated by federal law. The Standard is designed primarily for the health care industry, but also must be applied to research activities and practices which involve manipulation of certain human/NHP biological materials.

- If you can reasonably anticipate contact or risk of contact with human/NHP blood or other potentially infectious materials as a result of performing your research laboratory duties, the BBP Standard applies to you. In the research lab setting, the BBP Standard also applies to people who work in areas where these materials are manipulated by others.

7.4 The Exposure Control Plan Requirements and Guidance Table

- Employers are mandated by OSHA to provide an Exposure Control Plan (ECP) to employees with BBP exposure risks in their jobs. Exposure Control Plans are intended to provide information and promote work practices that will decrease/ eliminate occupational risks of BBP exposure. ECPs must include:
    - The specific BBP risks in manipulating biohazardous material, or performing particular tasks
    - The practices and controls in place to mitigate those risks
    - What to do in the event of an exposure, including how to report it

  - What to expect from an exposure investigation and follow-up
  - The types of records to be kept regarding BBP training, exposures, etc.
  - Where to find the ECP if employees need to refer to it

- Virginia Tech maintains a University Exposure Control Plan , which is a document used by facilities on campus that draw human blood and/or handle human clinical/diagnostic material. Because OSHA allows employers to include the content of an ECP in other documents (such as Biosafety Manuals), and because this content is now included in the *University Biosafety Manual* (UBM) and your completed *Lab-Specific Biosafety Manual* (LSBM), VT research laboratories do not have to maintain duplicate information in a separate ECP.

- **In reading this Manual and your LSBM, you will have reviewed all required topics, including lab-specific information. See the following guidance table for topic locations in each manual. The table is also provided in Appendix C of your LSBM.**

EXPOSURE CONTROL PLAN TOPICS	**READ ABOUT IT IN THESE SECTIONS OF THE *UNIVERSITY BIOSAFETY MANUAL* (UBM): (Crosslinked for ease of use.)**	**FIND DETAILS FOR YOUR LAB IN THESE SECTIONS OF THE *LAB-SPECIFIC BIOSAFETY MANUAL* (LSBM):**
BBP Risks for Lab Workers	**7.1**	
OSHA BBP Standard & Exposure Control Plan	**7.3**	
Universal Precautions	**4.1**	
Engineering Controls: • Handwashing facilities		**3.2**
• Needle safety; Sharps containment	**4.5**	**13.2**
• Biosafety cabinet containment	**4.16**	**11**
Personal Protective Equipment	**4.6**	**15**
Work Practices: • Handwashing	**4.4**	
• Sharps handling & disposal	**4.5**	**13.2**
• Avoiding aerosols	**4.22**	**13.1**
• Avoiding ingestion	**4.2 4.3**	
• Decontaminating surfaces	**4.12**	**14.1**
• Decontaminating equipment	**4.13**	
• Waste handling	**4.10**	**16**
Housekeeping Practices	**3.11**	**8**
Labels and Signs	**3.1**	
Occ. Health Medical Services • Hepatitis B vaccination	**7.5**	

• What constitutes an exposure	**7.6**	
• BBP exposure response	**7.7**	
• BBP exposure reporting	**7.8**	
• BBP exposure investigation & follow-up	**7.9**	
BBP Recordkeeping	**7.10**	
HIV/ HBV/ HCV Lab Practices	**7.11**	
BBP Definitions	**TERMS RELATED TO BLOODBORNE PATHOGENS**	

7.5 Hepatitis B Vaccination Program For Workers With BBP Exposure Risk

PURPOSE

- To provide and offer the Hepatitis B Vaccination to all employees *who have potential for an occupational exposure to bloodborne pathogens.*
- To provide testing, evaluation, and counseling to employees who have BBP exposure incidents.
- To document employee Hepatitis B vaccinations and declinations.

GENERAL

- All medical services will be provided at NO COST to the employee.
- The University will provide, through Environmental Health and Safety (EHS), all approved services.
- All medical services will be available at a reasonable time and place.

RECEIVING THE HEPATITIS B VACCINATION SERIES

- Employees must attend a BBP training class, or complete EHS *Biosafety for Research Labs* online training prior to requesting the vaccinations. Vaccinations will be provided to those who have never been vaccinated, and who express their intentions to receive this service on a written declaration (in class training) or through choosing that response option in the online training.
- Instructions for arranging for appointments for Hepatitis B vaccinations will be provided to these individuals.
- The series involves three separate injections, spaced at one and six month intervals.
- Employees may choose to decline the vaccinations; this must be document on a Declination Form (in class training) or by choosing that response option in the online training. If an employee declines the vaccination, but at a later date is still at risk for occupational exposure to BBP and decides to receive the vaccination series, he/she can contact EHS to expedite that request at no cost to the individu
- The U.S. Public Health Service currently does not recommend a routine booster for Hepatitis B.

7.6 What Constitutes A BBP Exposure

- When a potentially infectious material (human blood, tissue or other potentially infectious material) makes contact, or may have made contact with:
    - an injury site on your body (needlestick or cut)
    - a non-intact skin surface (chapped, abraded skin, open sores, dermatitis, etc.)
    - mucous membranes of your eyes, nose or mouth, this is considered a risky exposure.

- We must respond to BBP exposures in the ways described below.

7.7 What To Do In the Event of a BBP Exposure

- Remove PPE and provide immediate care to the exposed site by washing wounds and skin with soap and water for 15 minutes, or flush eyes/ mucous membranes with fresh water for 15 minutes.
- Call 911 if serious injury has occurred; administer first aid as needed.
- Inform your direct supervisor about the incident immediately, even if it is only a potential exposure.
- Visit your medical provider or an Emergency Department for evaluation within 1-2 hours of the incident. Inform your medical provider about the specific material to which you have been (or may have been) exposed.

7.8 Reporting A BBP Exposure

- Complete the Employers Accident Report.
- Contact EHS at 231-3600 during regular business hours; call 911 after hours, and the dispatcher will connect you with EHS personnel.
- Complete Exposure Incident Report Form and return it to EHS.
- Receive instructions from EHS regarding the Occupational Health Physician's recommended testing and treatment.

7.9 BBP Exposure Follow-Up

Following a BBP exposure incident, EHS will immediately arrange a confidential medical evaluation and follow up. Post-exposure services include:

- Documenting the route(s) of exposure, and the circumstances under which the exposure occurred.
- Identifying and documenting the source individual, unless it is infeasible or prohibited by law to do so.

- Testing the source individual's blood to determine HBV and HIV infectivity, if unknown. Consent of the source individual must be obtained prior to testing.
- Collecting and testing of the exposed employee's blood to determine serological status for HBV, HCV, and HIV. This collection must occur as soon as possible after exposure.
- Administering of post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service.
- Counseling
- Evaluating reported illnesses
- Conducting follow-up testing of exposed employee's blood

7.10 BBP Record Keeping

EHS will maintain records on each Virginia Tech employee with occupational exposure to human materials. Records will be maintained for the duration of the individual's employment at Virginia Tech, plus 30 years.

Employee written consent is necessary prior to release of any information. However, records will be made available to representatives of OSHA upon request.

Information maintained by EHS includes:

- Name of employee
- VT Identification Number of employee
- A copy of the employee's Hepatitis records, including:
  - Vaccination Records
  - Titer Results (if available)
  - Declination Forms
  - Attachments to Declination Forms
- Exposure Incident Report and Physician's Written Opinion forms, if applicable
- Copies of evaluation and testing results associated with an exposure incident
- Training records

7.11 HIV / HBV/ HCV Research Laboratory Practices

Labs working with the HIV/ AIDS virus, or with Hepatitis B or C viruses are required to use a number of special practices. These are discussed in detail in the **OSHA BBP Standard 1910.1030 (e)(1) through (e)(5)**; extra training requirements are discussed in **1930.1030 (g)(2)(ix)**. If your laboratory is engaged in research with these agents/materials, you must read these Standards and comply with them.

REFERENCES

- *Arthropod Containment Guidelines, Version 3.1*
- *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*
- *Biotoxins Management and Handling*; Safety Library; Division of Research Safety, University of Illinois www.drs.illinois.edu/SafetyLibrary/BiotoxinsManagementAndHandling
- *Care and Feeding of Your Thermo Scientific CO2 Incubator*, Gabby Downs, 2 Nov. 2011
- *Class II, Type C1 makes Biosafety Cabinet Selection Easy*, Brian Garrett, LEED Green Associate, November 27, 2014 http://www.labconco.com/news/class-ii-type-c1-makes-biosafety-cabinet-selecti
- *Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008*
- *International Society for the Advancement of Cytometry cell sorter biosafety standards, Kevin Holmes, 13 March 2014*
- *Laboratory Biosafety Manual*, World Health Organization, 3rd edition, 2004.
- *NIH Guidelines for Research Involving Recombinant DNA Molecules*
- Nuaire Technical Bulletin: GTB0277, Rev 1 August/ 2015, *Clarifying the "Type C1" Biosafety Cabinet*
- *Research Use of Biological Toxins and Venoms;* Vanderbilt EHS, Vanderbilt University http://www.safety.vanderbilt.edu/bio/bio-toxins.php
- *Storage of Living Cells in Culture, Frank P. Smione, Amer. Type Culture Collection, 1992*

FORMS AND TEMPLATES

1. Equipment Decontamination Form
2. Clearance for Lab Access Form
3. Notification of Toxin Transfer Form
4. Biohazardous Shipping Request Form
5. Laboratory Standard Operating Procedure (SOP) template
6. Training Forms:
    a. Biosafety Training Record for New Personnel
    b. Lab Topics Training Record
    c. Biohazard Awareness Training Record
10. Proficiency Checklist for Lab Personnel
11. Lab Incident Report Form