



The University Of

T A M P A

Biological Safety Program

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RECORD OF AMENDMENTS

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1 PURPOSE AND SCOPE

This document provides a guide to common practices related to working with biological materials in teaching and research laboratories at The University of Tampa [UT]. Laboratory Biological Safety [Biosafety] procedures are to be followed following registration and procurement of biological materials.

Biohazardous agents or "biohazards" are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. The risk can be direct through infection or indirect through damage to the environment. The United States Centers for Disease Control [CDC] categorize biological agents by assigning them into one of four hazard levels. Biosafety Level 1 and Biosafety Level 2 [BSL-2] agents that pose moderate hazards to personnel and the environment are the only agents allowed onsite at UT.

Federal and state government regulations, training, safe work practices, safety equipment, and personal protective equipment contained herein shall be followed to minimize risk and transmission of biological agents to the environment or another campus member. In addition, the Principal Investigator is responsible for ensuring that all United States Department of Agriculture [USDA] or CDC permits or applications are obtained.

UT requires all faculty members request pre-approval through the Chemical Hygiene and Biological Safety Officer [CHBO] before receiving biological agents onsite.

1.1 REGULATORY STANDARD

OSHA regulation 1910.1030 for Bloodborne Pathogens [BBP] in the workplace requires an Exposure Control Plan be acknowledged when working with biohazardous agents. The UT Bloodborne Pathogens Exposure Control Plan can be viewed on the UT Chemical Safety website <http://utweb.ut.edu/ehs> and is applicable to any research or teaching activity conducted with material derived from humans including blood, body fluids, tissues, and primary or established cell lines.

The CDC specified requirements for activities involving BSL-2 will also be followed and can be referenced in their document titled "*Biosafety in Microbiological and Biomedical Laboratories*" document on their website at:

http://www.cdc.gov/biosafety/publications/bmbl5/BMML5_sect_IV.pdf.

The National Institute of Health published [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#) to specify institutional oversight for safe handling and

containment and shall be incorporated even if research is not funded by NIH. The following incidents require reporting to the NIH Office of Biotechnology Activities [NIH OBA]:

Immediate Reporting

1. Spills or accidents involving rDNA requiring BSL2 containment resulting in an overt exposure, e.g. needle stick; splash in eyes, nose, mouth; or accidental aerosolization/inhalation;

Report within 30-Days

2. Any significant problems or violations of the NIH Guidelines, e.g. failure to adhere to the containment and biosafety practices; and
3. Any significant research-related accidents and illnesses, e.g. spill or accident leading to personal injury or illness or a breach in containment.

2 RESPONSIBILITIES

2.1 PRINCIPAL INVESTIGATOR

The Principal Investigator [PI] is the faculty member who is responsible for the biohazardous agents within the assigned laboratory. The PI is responsible for full compliance with the policies, practices and procedures set forth by the federal and state regulations and UT policies and procedures. This responsibility extends to all aspects of biosafety involving all individuals who enter or work in the PI's laboratory or collaborate in carrying out the PI's activities. Although the PI may choose to delegate aspects of the Biosafety Program in his/her laboratory to other laboratory personnel (laboratory directors or supervisors) or faculty, this does not absolve the PI of his/her ultimate responsibility. The PI remains accountable for all activities occurring in his/her laboratory. Documentation of training and compliance with appropriate biosafety practices and procedures are essential. The PI is responsible for assuring the appropriate safety training of employees and for correcting unsafe working conditions.

As part of general responsibilities the PI shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the requirements set forth by the *NIH Guidelines* and the CDC "Biosafety in Microbiological and Biomedical Laboratories" document as applicable.
2. Develop written laboratory-specific biosafety procedures that are consistent with the nature planned activities and make updates as necessary.

3. Select appropriate microbiological practices and laboratory techniques to be used for the research project or laboratory course.
4. Ensure that all laboratory personnel, including other faculty members, understand and comply with these laboratory-specific biosafety procedures.
5. Delay initiation of the project until all established safety equipment, policies and procedures have been implemented and confirmed by the CHBO.
6. Inform all laboratory personnel, maintenance personnel and visitors who may be exposed to any biohazardous agents are informed in advance of their potential risk and of the precautions required to minimize that risk.
7. Identify appropriate safety practices to those who may have potential exposure to biohazardous agents before they enter or work with such hazards.
8. Ensure that all maintenance work in, on or around contaminated equipment is conducted only after that equipment is properly decontaminated by the laboratory staff or PI.
9. Ensure that materials are properly decontaminated before disposal and that all faculty or laboratory personnel s are familiar with the appropriate methods of waste disposal.
10. Report any significant incidents, violations of the policies, practices and procedures to the CHBO as soon as reasonably possible.
11. Notify the CHBO immediately if:
 12. A laboratory-acquired infection is known or suspected.
 13. A spill of any quantity involving an agent infectious to humans, plants, or animals occurs.
 14. Provide evidence of training in standard microbiological techniques.
 15. Ensure that all affected personnel are appropriately trained in biosafety and receive appropriate medical surveillance when needed.
 16. Develop lab-specific emergency plans for handling accidental spills and personnel contamination.
 17. Encourage a laboratory environment that promotes open discussion of biosafety issues, problems and violations of procedure.
 18. Comply with shipping requirements for biohazardous agents and select agents to ensure that all applicable transportation safety regulations have been met prior to shipping microbiological cultures, tissues (human or animal) or body fluids.
 19. Complete and keep current any registration forms for the use of infectious agents, recombinant or synthetic nucleic acids and acute biological toxins.

The PI shall ensure the following activities are completed prior to initiating BSL-2 Research:

1. Describe the potential biohazards and the precautions to be taken to all laboratory staff and involved facilities staff.

2. Instruct and train all personnel in:
3. Identification of the biohazard(s) present;
4. Practices and techniques required to reduce potential exposure and ensure safety and,
5. Procedures for dealing with accidents, spills and exposures.
6. Inform the laboratory staff of precautionary medical practices advised or requested (e.g., vaccinations).
7. Do not ship any biohazardous agents without full disclosure to the recipient and complying with all applicable packaging and shipping requirements.
8. Maintain a formal inventory log of all biological material received that includes the approximate quantity of the materials and where it is stored in the laboratory.

PI continuous duties includes:

1. Supervise the laboratory staff to ensure that required safety practices are employed.
2. Investigate and report in writing to the CHBO any significant problems pertaining to the operation and implementation of containment practices and procedures.
3. Immediately notify the CHBO of any laboratory spills, accidents, containment failure or violations of biosafety practice which result in the release of biohazardous agents and/or the exposure of laboratory personnel (or the public) to infectious agents.
4. Correct work errors and conditions that may result in the release of biohazardous agents.
5. Ensure the integrity of all containment systems used in the project, lab, or course.
6. Restrict access as required by the laboratory-specific biosafety practices and procedures.

Failure to comply with the procedures in this manual and government regulations can result in severe consequences [i.e. disease, injury, or death]. The University will not tolerate noncompliance in these matters and expects full adherence to the strictest safety protocols.

3 BIOSAFETY RISK ASSESSMENT

A biosafety risk assessment is conducted to determine the appropriate containment for proposed research or laboratory coursework. There are four commonly recognized Risk Groups that should be determined for each selected biological agent in use.

SUMMARY OF RISK GROUPS (RG)

RG1	Agent not associated with disease in healthy adult humans; <i>B. subtilis</i> , <i>E. coli</i> K-12,
RG2	Associated with human disease which is rarely serious and preventive or therapeutic interventions are often available; Human adenoviruses, human herpesviruses (except herpes B), <i>Staphylococcus aureus</i> , amphotropic murine leukemia virus, influenza viruses type A, B, and C
RG3	Serious or lethal human disease; preventive or therapeutic interventions may be available; <i>Mycobacterium tuberculosis</i> ,
RG4	Serious or lethal human disease; preventive or therapeutic interventions are usually not available; Ebola, Marburg, Lassa, and Herpes B virus

There are also four biosafety levels that are established based upon the risk factor. The following sources should be consulted to determine the appropriate biosafety risk level of each biological agent:

1. The NIH Guidelines Appendix B provides [common biological agents used in research listed by Risk Group](#).
2. [Agent Summary Statements for some infectious agents](#) are provided in the Biosafety in Microbiological and Biomedical Laboratories [BMBL] and indicate the appropriate biosafety level for some infectious agents. Section II of the [BMBL] describes the process of Biological Risk Assessment.
3. The American Biological Safety Association [ABSA] website provides a [searchable database of many biological agents](#) and their assigned biosafety levels by country.
4. The [Pathogen Safety Data Sheets](#) are produced by the Public Health Agency of Canada as educational and informational resources for laboratory personnel working with certain infectious substances.

BSL	AGENT	PRACTICES	PRIMARY BARRIERS AND SAFETY	FACILITIES (SECONDARY BARRIERS)
1	Not known to cause disease in humans	Standard Microbiological Practices	Gloves, lab coat, eye protection , and	Handwashing sink, safety shower/eyewash

BSL	AGENT	PRACTICES	PRIMARY BARRIERS AND SAFETY	FACILITIES (SECONDARY BARRIERS)
2	Transmitted by percutaneous injury, ingestion, mucous membrane exposure. Consider aerosolization.	BSL-1 practice plus: <ul style="list-style-type: none"> • Restricted access • Biohazard signs • Biosafety manual that -defines “sharps” precautions, bio-waste practices, • Medical surveillance • Spill clean-up. 	At a minimum, BSL-1 protection, plus: Physical containment devices used for all manipulations requiring BSL-2 (microbes, rDNA, toxins) that cause splashes or	Same as BSL-1
3	Potential for aerosol transmission	BSL-2 practice plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum 	Primary barriers: <ul style="list-style-type: none"> • Class I or II BSCs or other physical containment devices used for all open manipulation of 	BSL-2 plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double- door access • Exhaust air
4	Not Permitted at UT			

The American Society of Microbiology has published the [ASM Guidelines for Biosafety in Teaching Laboratories](#) and this is commonly considered the standard of practice for all teaching courses and projects where students handle live microorganisms. Some important items to consider include:

- Instructors may not inquire about the health status of a student.
- Prior to beginning lab-work, all students must be provided with a list of all cultures (and their sources) used in the course.
- Students shall not subculture isolates from the environment at BSL-1. Unknown environmental microbes can be isolated and plated in a BSL-1 laboratory, but then the plates must be sealed and only observed.

3.1 Human Derived Components

Human blood, bodily fluids, tissues and cells require Biosafety Level 2 [BSL-2] practices and containment. Refer to [Appendix H](#) of the BMBL Working with Human. NHP and other Mammalian Cells and Tissues. In addition, the OSHA Bloodborne Pathogens [BBP] standard applies to all work in the laboratory with human blood or potentially infectious materials.

3.2 Other Cultured Cells and Tissue

Cultured cells which are known to contain or be contaminated with a biohazardous agent (e.g. bacteria or viral) are generally classified at the same risk group as the agent. Cell lines that are not human or other primate cells and which do not contain known human or zoonotic pathogens are often designated for work at Biosafety Level 1 [BSL-1]. However, these may require permits through [NCDACS](#) or the USDA (Principal Investigators are responsible for obtaining all necessary permits).

The following cells and tissue must be handled as BSL2:

- Human and non-human primate primary cells, established cell lines, and unfixed tissue;
- Cell lines exposed to or transformed by a human or primate oncogenic virus; and
- Cells, cell lines or tissue infected with pathogens requiring BSL-2 containment.

3.3 Arthropod Containment

The American Society of Tropical Medicine and Hygiene and the American Committee of Medical Entomology provide recommendations for arthropod Containment Levels 1-4 see [Arthropod Containment Levels \[ACLs\]](#). These guidelines specifically do not cover *Drosophila spp.* unless modified in such a manner that they would be of public health concern. Arthropods will be handled in minimum BSL-2 containment to minimize risks and reduce escape of exotic or genetically modified species.

Guidance for design, construction, maintenance and operation of facilities for containment of nonindigenous arthropod herbivores, parasitoids and predators which may be used in biological control research is provided in the USDA APHIS – PPQ [Guidelines for Containment of Nonindigenous Arthropod Herbivores, Parasitoids and Predators](#).

4 TRAINING

Laboratory personnel shall be trained to ensure they reduce inherent risks associated with hazardous agents. Training shall include general safety practices and task specific procedures as outlined in the Laboratory Specific Standard Operating Procedures. The following summarizes minimum training topics:

- Standard Operating Procedures
- Entry and Exit Procedures
- Use of PPE

- Safety Equipment
- Incident and Accident Reporting
- Emergency Actions

PIs are responsible for training and retraining new staff in practices to ensure laboratory techniques and safety precautions are well understood. For more information consult the [CDC Guidelines for Laboratory Biosafety Competency](#).

Training on the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is required by the NIH of all Principal Investigators who work with recombinant or synthetic nucleic acid molecules.

5 Medical Surveillance

5.1 Personal Health Status

Some individuals may be more susceptible to infection or be adversely impacted by immunizations or prophylactic interventions. Therefore, all laboratory workers and particularly women who may be or are planning to become pregnant should be provided with information regarding immune competence and conditions that may predispose them to infection such as known allergies to antibiotics or other chemo prophylactics that could be used to treat a laboratory acquired infection.

Lab workers that have an autoimmune or chronic disease (no matter how well managed), heart disease, are taking immune suppressing medications (e.g., chemotherapy, systemic steroids) or are otherwise immunocompromised (e.g., transplant recovery, cancer, lupus), should be encouraged to self-identify to the occupational healthcare provider for appropriate counseling and guidance.

5.2 Medical Surveillance Program

UT has a process for affected laboratory workers to be vaccinated for various infectious agents that may be used in the laboratory. Additionally, this program also provides for a post-exposure evaluation and follow-up examination by their designed Licensed Health Care Provider.

6 SAFETY EQUIPMENT

6.1 Biological Safety Cabinets

Biological safety cabinets [BSC] control airborne contaminants during work with infectious materials through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. The Class II BSC is the most commonly used BSC at UT and mandatory for all laboratory permitted for BSL-2 agents. Refer to [CDC's guidance document titled](#) : *Selection, Installation, and Use of Biological Safety Cabinets by the U.S. Department of Health and Human Services* for more information.

6.1.1 General Rules for Safe BSC Use

- Ensure BSC is annually tested and certified for use.
- Do not work in a BSC that is in alarm.
- The BSC should be located away for air supply grills, high traffic areas, and other equipment that causes turbulence.

Before beginning work within the BSC perform the following steps:

1. Monitor alarms, pressure gauges, or flow indicators for any changes.
2. Shut off the UV light.
3. Turn the cabinet on and let it run for 3-5 minutes.
4. Wipe work surface with an appropriate disinfectant.
5. Place a pan filled with disinfectant or lined with a small biohazard bag inside the BSC to collect discards. Avoid reaching outside of the BSC during procedures to discard waste in floor containers.
6. Plan your work and place everything needed for the procedure, including the pan for your discards, inside the BSC. Wipe items with disinfectant before placing in BSC.

BSC Safe Use Tips

7. Keep the BSC free of clutter, e.g. extra equipment and supplies
8. Don't place objects over the front air intake grille.
9. Don't block the rear air intake grille.
10. Limit traffic in the area when the BSC is in use
11. Make sure lab door is closed, and avoid opening and closing door if located near the BSC.
12. Move arms slowly when removing or introducing items.
13. Keep all materials at least 4 inches inside the sash.

14. Place a centrifuge or blender that creates air turbulence in the back 1/3 of the cabinet and stop other work while the equipment is running.
15. Don't operate a Bunsen burner in the cabinet.

BSC Working Tips

1. Work as far to the back of the BSC workspace as possible.
2. Segregate contaminated and clean items. Work from "clean to dirty."
3. Clean up all spills in the cabinet immediately. Allow cabinet to run for 3-5 minutes before resuming work.

After SBC Work Tasks:

1. Wipe down all items with an appropriate disinfectant before removing. Remove all materials and wipe all interior surfaces with an appropriate disinfectant.
2. Periodically decontaminate under work grilles.

6.1.2 Decontamination

Gaseous decontamination is mandatory when required maintenance work, filter changes, and performance tests require interior access. The following tasks should be performed during the decontamination process:

1. Contact the CHBO for a mandatory room air pressure differential test.
2. Disinfect and remove all items from within the BSC.
3. Surface disinfect the interior of the BSC w/ appropriate disinfectant.
4. Schedule a full gas decontamination (must be conducted by a BSC certification vendor, typically the same professionals conducting annual certification).
5. Contact building liaison to ensure no HVAC disruption is scheduled at that time because the gas decontamination procedure uses high concentrations of toxic gas.
6. Schedule for lab to be vacant during the gas decontamination procedure.
7. Post "DO NOT ENTER" signs at entryways to the gas decontamination area.
8. After gas decontamination, remove/cover biohazard stickers
9. Ensure the gas decontamination vendor posts a label/sign indicating the following:
 - a) Vendor contact information
 - b) Date and time the gas decontamination was performed
 - c) Method used for gas decontamination
 - d) If the gas decontamination method was successful or not

10. Contact building liaison to disconnect gas lines, vacuum, etc. from BSC
11. If exhaust is hard-ducted, the duct will need to be disconnected

6.2 Aerosol-Proof Equipment

The use of certain devices, e.g. blenders, homogenizers, sonicators (ultrasonic disrupters) can produce aerosols. To reduce exposure to aerosols, these devices should be used in a biosafety cabinet whenever possible.

Safety blenders and the [BeadBeater homogenizer \(BioSpec Products\)](#) are designed to prevent leakage of aerosols. The devices should be used in the BSC to prevent accidental release of aerosols.

Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes splitting or breaking. Follow the procedures below when centrifuging biohazardous materials:

1. Use aerosol-proof rotors or safety buckets with caps that seal with O-rings.
2. Before use inspect O-rings and safety caps for cracks, chips, and erosion.
3. Use tubes with threaded caps. Avoid overfilling the tube and getting caps/closures wet. Wipe tubes down with disinfectant after filling.
4. Load and unload rotors and buckets inside the BSC
5. Balance buckets, tubes and rotors before centrifuging.
6. Disinfect the centrifuge after use.
7. Place small, low-speed centrifuges in a BSC during use to contain aerosols.

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable loops and needles can be used.

7 SAFE WORK PRACTICES

Principal Investigators are responsible for ensuring that proper safety practices, procedures, and equipment are in place. OSHA considers the PI as the responsible “supervisor” regardless of whom they delegate these tasks to. Supervisors are responsible for conducting workplace assessments and to select and train employees in the proper use of PPE (e.g., lab coats, gloves, safety glasses, face shields, etc.). A workplace assessment may be conducted

during protocol review, at lab meetings, or while mentoring novel techniques and practices. This assessment should be documented and, if necessary, practices written up as a safety SOP.

Proper work practices protect you and others from exposure to infectious materials, reduce the possibility of cross-contamination, and improve the quality of the work performed.

1. Label all equipment used to store infectious materials with a biohazard warning label.
2. Keep an uncluttered work space
3. Plan work procedures with safety in mind
4. Remove PPE and wash hands when leaving the lab
5. Don't eat, drink, smoke, apply cosmetics, and handle contact lenses in the lab
6. Don't mouth pipette
7. Decontaminate work surfaces at the end of an experiment and after a spill occurs
8. Decontaminate reusable PPE as soon as possible after it has been contaminated. Lab coats can be spot treated with 10% bleach or autoclaved before laundry. Never take lab coats home.
9. Protect house vacuum lines and vacuum pumps by using a hydrophobic HEPA filter installed between the collection flask and vacuum source
10. Change gloves often and as soon as possible when visibly contaminated
11. Minimize aerosol production by working carefully
12. Perform procedures that may result in aerosols or splashes in a BSC
13. Use aerosol-proof rotors or safety cups when centrifuging and load and unload them in a BSC

7.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) is specialized clothing or equipment worn by laboratory personnel for protection against hazards. PPE should be worn while working in the laboratory and must not be taken home or worn outside the laboratory in non-laboratory areas. Contact the CHBO For assistance in selecting PPE.

BSL-1 and BSL-2 laboratories require the minimum PPE: lab coats, gloves, and safety glasses (or goggles).

1. Laboratory garments, e.g. lab coats, scrubs, and gowns, should be long-sleeved in order to prevent contamination of skin and street clothes. The garment must be fluid-resistant

in order to protect workers from splashes. Lab coats should be provided for visitors, maintenance and service workers as needed.

2. Gloves must be worn when working with biohazards. Temperature resistant gloves must be worn when handling hot material or dry ice. If personnel develop or have latex allergies, then nitrile gloves should be used in the lab with biohazards instead of latex gloves. Gloves should overlap the sleeve of the lab garment. Double-gloving adds further protection and is recommended in some circumstances where a spill or splash is possible.
3. Face protection is required in situations where chemical splashes or aerosol exposure to infectious material are possible. Goggles or safety glasses with side shields should be used in combination with masks, face shields or other splatter guards for optimal protection.
4. Respirators may be necessary in some cases. Personnel who require respiratory protection must be evaluated by the UT designated Licensed Health Care Provider and trained in respirator selection and usage. Personnel required to wear tight-fitting respirators must enroll in UT's [Respirator Protection Program](#).

7.2 SHARPS PRECAUTIONS

Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of injuries from sharps. The following list of precautions must be followed when working with sharp items:

1. Use of needles and other sharps should be minimized and avoided entirely whenever possible. Many glass items such as Pasteur pipettes have plastic counterparts that are safer alternatives.
2. If the use of sharps is necessary, extra precautions should be taken. Sharps should be disposed of immediately after use in a designated puncture-resistant sharps container. When the container is 2/3 full contact the CHBO for its removal. Never allow the container to overfill.
3. Needles must never be recapped, removed from the syringe, sheared, bent or broken. If a needle must be recapped, use a one-handed method or a mechanical device, e.g. forceps.
4. Use a mechanical device to remove scalpel blades, and not your fingers.
5. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

Biohazard sharps waste is material used with rDNA, BSL-1, or BSL-2 material that have sharp edges capable of causing punctures or cuts, including, but not limited to the following: needles, syringes, scalpels, razor blades, slides, coverslips, Pasteur pipettes, capillary tubes, and broken glass and plastic. Plastic serological pipettes are considered “sharps waste” if they are broken and have a sharp edge.

1. Labs collect biohazard sharps waste in labeled plastic sharps containers. To avoid injury, do NOT clip, bend, shear, or separate needles from syringes and do NOT recap needles.
2. When the container is $\frac{3}{4}$ full, cap it, autoclave as applicable. Do not overfill the sharps waste container.
3. Contact CHBO for disposal information

7.3 DOOR SIGN

BSL-2 door sign information contains the universal biohazard symbol, biosafety level, and office and after-hours contact numbers for the PI and alternate who is familiar with the laboratory hazards and operations. Additional placards for BSL-2 areas may be required if additional information such as PPE and enhanced warnings are necessary.

Door signs and placards need not be posted for laboratories where containment is limited only to BSL-1 or BL-2P (plant) work.

7.4 DISINFECTION

Characteristics of microorganisms affect their resistance to disinfection. The table below provides a starting point for identifying appropriate chemicals for disinfection depending on the circumstances and type of biohazard. To locate information on proprietary disinfectants, refer to the EPA-registered disinfectants website at <http://www.epa.gov/oppad001/chemregindex.htm> to review efficacy claims against microbes of interest.

OSHA requires use of an EPA-registered disinfectant under the Bloodborne Pathogens Standard. Note that **70% ethanol is not an EPA-registered disinfectant**. It evaporates too quickly to be an effective disinfectant. 70% ethanol can be used as a cleaner, for example, to remove excess bleach or other EPA-registered disinfectants. Alternative disinfectants include Clorox, Amphyl, Lysol, and Sporidicin.

At UT liquid biohazard waste is autoclaved with a test indicator and disposed down the sanitary sewer. Chemicals may NOT be directly poured down the drain. For example, any greater concentration than a 1:5 dilution of bleach (5.25% sodium hypochlorite) to the final volume needs review of disposal options with the CHBO. If chemicals are to be used to disinfect liquid

media, etc., the final waste product must adhere to all chemical waste disposal regulations. For suction flasks, make sure the approved chemical disinfectant is in the flask *before* suctioning off the media.

When decontaminating small tubes such as epi tubes, empty them out into a plastic container in a sink, add a 1:10 dilution of household bleach (5.25% sodium hypochlorite) to water or another IBC approved disinfecting solution. After the appropriate contact time has been achieved (this is listed on the BUA), it may then be poured down the drain.

*LIST OF DISINFECTANTS**

	Ethylene Oxide	Paraformaldehyde (gas)	Quaternary Ammonium Cmpds.	Phenolic Cmpds.	Chlorine Cmpds.	Iodophor Cmpds.	Alcohol (ethyl or isopropyl)	Formaldehyde	Glutaraldehyde
USE PARAMETERS									
Conc. of active ingredient	400-800 mg/liter	0.3 g/ft³	0.1-2%	0.2-3%	0.01-5%	0.47%	70-85%	4-8%	2%
Temp. (°C)	35-60	>23							
Relative humidity (%)	30-60	>60							
Contact time (min.)	105-240	60-180	10-30	10-30	10-30	10-30	10-30	10-30	10-600
EFFECTIVE AGAINST									
Vegetative Bacteria	+	+	+	+	+	+	+	+	+
Bacterial Spores	+	+			±			±	+
Lipo Viruses	+	+	+	+	+	+	+	+	+
Hydrophilic viruses	+	+		±	+	±	±	+	+
Tubercle bacilli	+	+		+	+	+		+	+
HIV	+	+	+	+	+	+	+	+	+
HBV	+	+		±	+	±	±	+	+
APPLICATIONS									
Contaminated liquid discard					+			±	
Contaminated glassware	±		+	+	+		+	±	+
Contaminated instruments	±			+				±	+
Equipment total decontamination	±	+							

*These chemical disinfection methods are recognized by the National Institutes of Health, the CDC, or the American Biological Safety Association.

+ denotes very positive response
± denotes a less positive response
blank denotes a negative response or not applicable

8 BIOHAZARD WASTE MANAGEMENT

All biohazard waste generated in UT research, diagnostic, and/or teaching laboratories must be properly treated prior to its disposal.

- Materials contaminated or potentially contaminated during the manipulation or clean-up of material generated during research, diagnostic, and/or teaching activities requiring BSL-1 or BSL-2 or animal or plant or liquid blood and body fluids.
- Materials contaminated with human/primate tissue or human/primate tissue cultures (primary and established) because these are handled at BSL-2
- Animal blood, fluids and bedding from animals infected with BSL2 agents.

If treatment of waste is not an option contact CHBO for a biohazardous waste collection request.

8.1 SOLID BIOHAZARDOUS WASTE COLLECTION

1. Biohazard waste handling and treatment should only be performed by trained workers.
2. Collect BSL-1 and BSL-2 waste in red, hard-walled biohazard waste collection containers not to exceed 15 gallons and lined with a clear autoclavable bag. The lid must remain on the container when not in use (e.g., overnight, etc.). The lid and container each must bear the biohazard symbol with the “Biohazard”.
3. Autoclave bags must be clear and also have the biohazard symbol and the word “Biohazard” on the outside of the bag. Orange or red colored bags are not to be used. Each bag must be labeled with the first and last name of the Principal Investigator.
4. Bags must be removed from collection containers prior to being 2/3 full to allow headspace to seal the bag for transport to the autoclave. Never overfill biohazard waste collection containers. Place bags directly into secondary containers to contain spills. For dense or dry loads, add 200 mL of water to the bag to ensure steam penetration. Use only lead-free autoclave tape ([click the link](#) for more information on hazardous waste requirements).
5. A log detailing autoclave performance verification must be completed on every load and maintained at the autoclave. See below for Autoclave Performance Verification details.
6. Place all items in heat-resistant secondary containers to secure and contain spills. Bags should be opened before autoclaving to insure sterilization.

8.1.1 Autoclave Danger!

No one should use an autoclave unless they have received recent instruction in procedure for the specific autoclave unit they are operating or are working under the direct supervision of a user experienced with the specific autoclave unit they are operating.

8.1.2 Autoclave Emissions

Appropriate ventilation systems should be operating in areas where autoclaves are located. Avoid placing hazardous chemicals in the autoclave. For more information, read Hadar, Julia et al. 'Autoclave Emissions-Hazardous Or Not'. Journal of the American Biological Safety Association 2.3 (1997): 44-51 or contact the CHBO.

8.1.3 Unloading the Autoclave

The greatest risk of personal injury occurs during the process of unloading the autoclave. When the pressure gauge reaches zero, wait one to two minutes before opening the autoclave. It is dangerous to begin opening the autoclave before the pressure gauge reaches zero.

Minimum PPE for unloading an autoclave include long-sleeved heat-insulating gloves (these gloves are compromised if wet or have holes), lab coat, eye protection, and proper shoes. A rubber apron and face shield may also be worn.

1. Ensure cycle has completed and both temperature and pressure have returned to a safe range.
2. Wearing PPE, stand back from the door as a precaution and carefully open the door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.
3. Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
4. Do not agitate containers of super-heated liquids or remove caps before unloading.
5. Wear PPE to remove items from the autoclave and place them in an area which clearly indicates the items are "hot" until the items cool to room temp.
6. Shut the autoclave door.
7. Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.
8. Any breakage of bags or leakage of contaminated materials should be reported to the laboratory director or supervisor at once for instructions on procedures for safe cleanup.
9. Reseal the bags with tape and remove from the building.

8.1.4 Autoclave Performance Verification

All biohazard waste should be treated for a minimum of 45 minutes at 121°C (250°F) at 15 psi. Each load of biohazardous waste processed in an autoclave must meet the following conditions:

1. The operator will incorporate with each load a Chemical Integrator Test Pack (CITP), evaluate the performance of the autoclave based on color changed of the CITP; and document the results in a User Log. All bags autoclaved with a failed CITP will be autoclaved again. 3M SteriGage Test Packs #41360 is currently the system accepted for this test.
2. Users should make sure that the autoclave is working properly before re-autoclaving. If the autoclave needs repair a tag “Out of Service” must be placed on the autoclave.
3. Monthly, a biological challenge will be performed with a standard load. The biological challenge needs to be incubated for 48 hours. Test results will be documented in log [date tested, initial of person doing test; test results].

8.1.5 Liquid Biohazard Waste

The preferred method for disinfecting rDNA, BSL-1 and BSL-2 liquid waste for drain disposal is autoclaving on the liquid cycle. If the liquid waste was used for propagating microbes, viral vectors, or toxins, chemical disinfection followed by drain disposal must be listed on your Laboratory Specific SOP and approved by the CHBO.

8.1.6 Mixed waste:

Mixed waste often requires special procedures. Please contact the CHBO for proper disposal procedures.

1. Mixed biological/chemical waste can be disinfected by using carefully selected chemical treatments only if compatible with the other chemicals in the experiment. Handle resulting waste as hazardous chemical liquid waste.
2. Treat animal or human tissue in 10% formalin waste as liquid chemical waste and label the hazardous waste tag “10% formalin + non-infectious animal tissue” or “10% formalin + non-infectious human tissue.”.
3. Disinfect biologically contaminated radiological solid waste by soaking in a suitable disinfectant. Discard disinfectant waste in designated and posted sink if radiological contamination is within sink disposal limits.

4. Disinfect iodinated liquid waste with a phenolic disinfectant; e.g., Lysol™. Disinfect all other liquid waste with bleach (10% final concentration with 5.25% sodium hypochlorite.) If the waste is within radiological sink disposal limits, dispose of in designated and posted sink. If levels are above sink disposal limits, then package for hazardous waste collection.

9 EMERGENCIES AND INCIDENT REPORTING

Each laboratory with an approved Biological Use Authorization is required to have an Emergency Plan that includes documentation how to cease, terminate and secure the laboratory in case of a lab or campus emergency; a spill response plan specific to the agents used in the lab; and reporting requirements to CHBO in case of incident. The Emergency plan should be posted in the laboratory and reviewed by all lab occupants on an annual basis. The Principal Investigator shall keep written records to document that all occupants have been trained in the Plan.

A biosafety incident or “release” is either an exposure to personnel or an event that results in the discharge of a bioagent outside the primary containment barrier. The event can be the result of a splash or spill of infectious material or a failure of the containment system.

All spills, releases, or accidents involving materials registered on a Biological Use Authorization, regardless of how minor the event or how remote the location, must be reported to the CHBO.

An incident should be reported even if there is no obvious exposure resulting from the release. Laboratory acquired infections are often not associated with an apparent exposure. It is possible that lab workers exposed to volumes and concentrations of microorganisms not typically found in nature can present with atypical signs and symptoms.

As a minimum, each Emergency Plan should include the following if applicable to the respective laboratory:

9.1 MEDICAL EMERGENCY OR INJURY

OBTAIN MEDICAL ATTENTION

- For serious medical emergencies day or night, dial 911.
- Minor injuries -- Notify your supervisor.

- Minor injuries – students: notify supervisor and report to Student Health Services.

ASSISTING IN MEDICAL EMERGENCY OR PERSONAL INJURY

- Dial 911 from a campus phone or cell phone.
- Do not move injured person unless there is a danger of further harm from remaining in the location. If the area is unsafe, then evacuate, close doors to area, and prevent access. Provide information to emergency responders.
- Remain with the injured person until medical assistance arrives. Initiate life-saving measures if necessary and if you have received appropriate training.

9.2 HAZARDOUS MATERIAL ON SKIN OR SPLASHED IN EYE

- Remove contaminated clothing, shoes, jewelry, etc.
- Immediately flood exposed areas with water from safety shower, eyewash, or faucet for at least 15 minutes (use soap on skin for biological/blood exposure). Hold eyes open to ensure effective rinsing behind both eyelids.
- Immediately after rinsing, Obtain Medical Attention.
- After emergency telephone notifications are made, report the incident using Accident Incident Investigation Procedure and notify the CHBO.

9.3 NEEDLE STICK OR CUT WITH CONTAMINATED SHARP ITEM

- Immediately wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, OBTAIN MEDICAL ATTENTION.
- If sharps or needles are used in the lab follow BBP Policy.

9.4 Spill procedures for biohazardous material

If the spill cannot be handled safely by laboratory employees with available absorbents and disinfectants, notify your supervisor and/or UT Security 813-257-7777 to request assistance.

9.4.1 SMALL SPILL INSIDE BIOSAFETY CABINET:

1. Contain spill with absorbent paper.
2. Dampen paper with disinfectant. Allow to stand for 20 minutes.
3. If sharps/glass are present, use mechanical means to collect the waste (e.g. forceps, cardboard flaps).
4. Remove gloves after area is decontaminated.
5. Wash hands.

9.4.2 LARGE SPILL INSIDE BIOSAFETY CABINET:

1. If splash has occurred outside the cabinet resulting in personnel exposure to infectious material, the Principal Investigator and CHBO should be notified immediately and the need for prophylactic treatment or other medical attention determined.
2. Contaminated clothing should be removed and containerized for autoclaving.
3. Thoroughly wash hands and face, if exposure has occurred.
4. Remove gloves after area is decontaminated
5. Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.
6. Spray or wipe walls, work surfaces, and equipment with appropriate disinfectant.
7. Flood top tray, drain pans, and catch basin below work surfaces with disinfectant and allow to stand 20 minutes. Dump excess disinfectant from tray and drain pans into cabinet base.
8. Lift out tray and removable exhaust grille work. Wipe off top and bottom (underside) surfaces with disinfectant sponge or cloth. Replace in position.
9. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.
10. Drain disinfectant from cabinet base into an appropriate container and autoclave.
11. Remove gloves and wash hands.
12. This procedure does not decontaminate the interior parts of the cabinet such as the filters, blowers, and air ducts. If the entire cabinet is to be decontaminated with toxic gas refer, contact the CHBO.

9.4.3 SPILL OUTSIDE BSC:

1. Decontaminate and/or remove all personnel, clothing and exit laboratory.
2. Wash hands and any exposed skin thoroughly.
3. Alert others in the area. Notify PI and UT Security at 813-257-777 if assistance is required.
4. If necessary, allow aerosols to settle for 30 minutes.
5. Re-enter wearing PPE (gloves, lab coat, and eye/face protection).
6. Cover spill with paper towels and carefully pour disinfectant, e.g., 10% bleach, around and over the spill from outside edges.
7. Allow contact time for disinfectant (e.g. 10% bleach for 20 mins).
8. Clean-up with paper towels. Pick up sharp items, e.g., broken glass or needles, with forceps or dust pan and brush and place in a sharps container.

9. Decontaminate or dispose of clean-up materials in biohazard bag.
10. Remove contaminated PPE and wash hands.

10 SHIPPING BIOLOGIC MATERIALS

The US Department of Transportation is vigilantly reviewing companies, carriers, and academic settings for compliance in packaging and shipping hazardous materials. It is the responsibility of individual shippers to properly identify their material and package it accordingly.

If you are transporting the materials yourself in a vehicle, even across campus:

- Notify CHBO for approval prior to initial transport
- Use a University Vehicle
- Don't contaminate the vehicle if collecting specimens.
- Check packaging to ensure sealed secondary container with Biohazard label on the outside
- Depending on volume, a spill clean-up kit may be required.

10.1 Training

Most biological materials require specific packaging, labeling, and documentation. Infectious materials (materials containing or expected to contain pathogens affecting humans) are regulated by the US Department of Transportation (DOT) and the International Air Transport Association (IATA). You must complete a hazardous materials shipping training course to be certified to ship infectious biological materials. This training is also required to be able to properly identify your materials according to DOT and IATA guidelines.

10.2 Import and Transfer Permits

Some biological materials require a permit to be imported or transferred to another institution outside of UT. The importation or interstate transfer of an etiological agent and hosts or vectors of human disease require an import permit from the Center for Disease Control (CDC) Etiological Agent Import and Interstate Transfers. This permit applies to the etiological agents themselves, unsterilized biological material (ex: patient samples) containing an etiological agent, and animals that could be a host or vector of disease in humans.

The United States Department of Agriculture (USDA) requires a permit for import or interstate transfer of infectious materials affecting livestock and biological materials containing animal material. Tissue culture materials and suspensions of cell culture grown viruses or other etiological agents containing growth stimulants of bovine or other livestock origins are

controlled by the USDA due to the potential risk of introducing exotic animal diseases into the US. For more information, review the Guide to USDA Animal and Plant Health Inspection Service (APHIS) Permits.

The U.S. Fish and Wildlife Service require an import permit for certain live animals. US Fish and Wildlife Services Permits: <http://www.fws.gov/permits/ImportExport/ImportExport.html>

If you are sending a material that requires an import or transfer permit it is your responsibility to ensure the recipient has the proper permits to receive the material before shipping the materials.

10.3 Export Licenses

Some pathogens, toxins, and genetically modified organisms require government licenses in order to be legally exported. The Department of Commerce and Department of State regulate the export of some biological materials, chemicals, and equipment. Do not assume that you will not need an export license based on the item's availability in the US. Failure to obtain an export license when one is needed can result in significant fines, loss of export privileges, or jail time.

10.4 Select Agent Transfers

All movements of Select Agents need to be approved and documented even if it is within the University. Contact CHBO if you are considering bringing in a Select Agent, shipping one outside of the University, or moving one from one location on campus to another.

11 General Biosafety References

[American Biological Safety Association](#) *Biosafety Links*

[Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#), National Institutes of Health, March 2013

[Biosafety in Microbiological and Biomedical Laboratories \(BMBL\), 5th Edition](#), Centers for Disease Control and Prevention, National Institutes of Health, February 2007

[Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets](#), BMBL Appendix A, Centers for Disease Control and Prevention, National Institutes of Health

[Federal Select Agent Program](#), Animal and Plant Health Inspection Service (APHIS) and the Centers for Disease Control and Prevention (CDC)



**Laboratory Biological
Safety Program**
Effective January 2015

Select Agents and Toxins List

Bloodborne Pathogens Standard CFR 1910.1030, Occupational Safety and Health Administration, U.S. Department of Labor