BIOSAFETY MANUAL

Office of Environmental Health & Safety

Boston, Massachusetts

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Emergency Contacts Information

Suffolk University Police Department (SUPD)	8111 or 8333	(617) 573-8111	N/A
Boston EMS/Fire/Police	911	911	911
Office of Environmental Health & Safety (OEHS)	X4849 X8628	(617) 570-4849 (617) 573- 8628	(617) 947-8573 (857) 330- 0914
Facilities Management	8110	(617) 573-8110	Call SUPD

Introduction

The purpose of this manual is to serve as a resource for researchers and staff and to encourage and give support to their activities in a manner that:
Protects all University personnel and visitors from laboratory-acquired infections;
Maintains the security and integrity of specimens and other research materials;
Provides environmental protection to minimize risks to those outside the laboratory and beyond the confines of the campus; and,
Ensures compliance with existing Federal, State, and City health, safety, and environmental regulations and guidelines.
Whenever there is additional activity- or agent-specific information is required, Office of EHS (OEHS) will assist investigators in developing and implementing appropriate practices to minimize the risk of laboratory infection or environmental contamination because no single document can address every contingency. When indicated in future expertise of an Institutional Biosafety Committee or other resources may be called upon for additional input.
Principal Investigators are responsible for seeking out these and other resources. They must also ensure that all personnel under their supervision are appropriately trained, informed of applicable regulations and guidelines and that they are capable, based on academic background and hands-on experience, of working within these regulations and guidelines. The PI and lab managers are responsible for ensuring there is full compliance to this manual and that they will use this manual as a starting point for their Biosafety compliance.
Biological Safety is a dynamic field; researchers at times use organisms that were not even identified a few years ago and the technology (and the nature of the hazards) associated with them is continually evolving. Between periodic revisions of this manual, OEHS will continue to communicate pertinent biosafety information to the research community via e-mail, trainings, newsletters and other media, and during periodic laboratory surveys.
OEHS is responsible for the implementation of this manual whenever it applies on Campus however, individual departments or researchers are responsible for complying and updating their respective SOPs that need to be appended and reaching out to OEHS whenever there is need for update to this general guidelines based on their work if that update is not specific to a single SOP.

Rules, Regulations & Guidelines

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and recombinant DNA molecules. The copies of these documents are available on the agencies websites.

1. **Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) Guidelines on: Biosafety in Microbiological and Biomedical Laboratories, Fifth Edition (BMBL5)**. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. The BMBL has been revised several times and is commonly seen as the standard for biosafety. Suffolk University is using the BMBL as the basis for this biosafety manual.

2. **National Institutes of Health (NIH): Guidelines for Research Involving Recombinant DNA Molecules**. These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA molecules and organisms containing them. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed research using the NIH Guidelines as a minimum standard. For more information, please refer to the following section of this manual: *Biosafety and Recombinant DNA Technology,* the NIH Guidelines for Research Involving Recombinant DNA Molecules.

3. **Occupational Safety and Health Administration: Bloodborne Infectious Disease Standard**. In 1992, the Occupational Safety and Health Administration (OSHA) promulgated a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions. Consequently, Suffolk University established an Exposure Control Plan to protect employees from exposure to HIV, Hepatitis B and other bloodborne pathogens. For more information, refer to the SU Exposure Control Plan or your labs exposure control plan when available.

4. **Massachusetts Department of Public Health: Medical Waste:** Regulation 105 CMR 480.000 Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code, Chapter VIII).

5. **Packaging, shipment and transportation requirements** for infectious substances, diagnostic specimens, biological products and genetically modified organisms are addressed in the following rules and guidelines:

- United Nations
  Recommendations of the Committee of Experts on the Transportation of Dangerous Goods
- International Civil Aviation Organization (ICAO)
  Technical Instructions for the Safe Transport of Dangerous Goods by Air
- International Air Transport Association (IATA)
  Dangerous Goods Regulations
- U.S. Department of Transportation
  49 CFR Parts 171-178
- U.S. Public Health Service
  42 CFR Part 72
- U.S. Postal Service
  39 CFR Part 111
- U.S. Department of Labor, OSHA
  29 CFR 1910.1030

6. Permits are required for importation of certain infectious agents, biological materials

and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

7. The City of Boston requires that all nucleic acid work within their boundaries be registered with the Boston Public Health Commission Board of Health and comply with CDC/NIH guidelines.

Risk Assessment

Risk is the probability that harm, injury, or, in the context of this document, disease will occur. The foundation of any safety program is the use of control measures appropriate for the risk posed by the activities and the agents in use. To characterize their risk, microorganisms and clinical materials are assigned to one of four Biosafety Levels (BSLs). For each BSL there is a unique set of safety equipment, facility design features, and practices that will reduce the risk of laboratory-acquired infections.

A complete description of work practices, safety equipment, and facility design features for BSL-1 through BSL-4 is available in the CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (BMBL5), Section IV.

The following excerpts are general summaries and users are required to review the more comprehensive information in the BMBL5. The NIH's Guidelines for Research Involving Recombinant DNA Molecules provide additional information along these lines as well as guidance for risk assessment of microorganisms and materials containing recombinant DNA, which may add or reduce the risk of the research.

Biosafety Level 1 (BSL-1)

- Agents: defined and characterized strains of microorganisms not known to consistently cause disease in healthy adults e.g., *B. subtilis*, *S. cerevesiae*, non-pathogenic *E. coli*. Includes recombinant DNA activities using such non-pathogenic organisms as hosts for the expression of genes incorporated into bacterial plasmids or low risk viral vectors such as baculovirus or Adeno Associated Virus.
- Work practices: standard microbiological practices/aseptic technique.
- Safety equipment: gloves, lab coats and eye protection recommended.
- Facilities: bench top sink available for hand washing.

Biosafety Level 2 (BSL-2)

- Agents: associated with human diseases of varying severity, e.g., Hepatitis B and C, HIV, *S. typhi*, human retroviruses, *S. aureus*. Includes recombinant DNA activities using viral vector systems such as Adenoviruses and some Retroviral

vectors, particularly Lentiviral vectors, and expression of recombinant DNA in BSL-2 organisms.
- Transmission: inoculation and other percutaneous injuries, ingestion, mucous membrane exposure
- Work practices: BSL-1 practices, with the addition of: limited access, 'Biohazard' signs, 'sharps' precautions, defined procedures for Regulated Medical Waste (RMW) disposal and medical surveillance as advised.
- Safety equipment: Class I or II Biological Safety Cabinet (BSC) or equivalent containment for manipulations with potential for aerosolization or splashing; lab coats, gloves, eye/face protection.
- Facilities: BSL-1 facilities, with the addition of: available autoclave, directional airflow, no air recirculation, disinfection/decontamination procedures in place.

Biosafety Level 3 (BSL-3) and Level 4 (BSL-4) None permitted at Suffolk University

Classification of Infectious Agents on the Basis of Hazard (Risk Groups)

Risk groups (RG) are a method used by the World Health Organization (WHO) and by the National Institutes of Health (NIH) to classify human etiological agents based on hazard to both the individual and to the community. There are four risk groups. These correlate to but are not equivalent to biosafety levels. Determining the risk group of a biological agent can be part of the biosafety risk assessment and helps in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BSL- 2, and RG-3 agents at BSL-3. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions, while some RG-3 agents may be safely manipulated at a BSL-2 under certain conditions.

Basis for the Classification of Biohazardous Agents by Risk Group

Risk Group	Risk to the individual and the community
Risk Group 1 (RG-1)	Agents that are not associated with disease in healthy adult humans (no or low individual and community risk).
Risk Group 2 (RG-2)	Agents that are associated with human disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).
Risk Group 3 (RG-3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
Risk Group 4 (RG-4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Examples of RG-1 agents include microorganisms like Escherichia coli-K12 or Saccharomyces cerevisiae. It is important to note however, that no list is all inclusive. Also, those agents not listed in RG-2, RG-3 or RG-4 are not automatically classified in

RG-1. Those unlisted agents need to be subjected to a risk assessment based on the known and potential properties of the agents.

Biosafety Level classifications are appropriate for typical laboratory operations. The Principal Investigator or laboratory manager is responsible for implementing more (or less) stringent practices based on laboratory specific conditions. Such a decision is ultimately the result a risk assessment process that accounts for the following:

- Pathogenicity - the ability of an organism to cause disease.
- Virulence - the severity of disease.
- Transmission route - parenteral, ingestion, mucous membrane exposure, or inhalation. The latter route is of the greatest concern which is why organisms such as M. tuberculosis require more stringent control than organisms that are transmitted via direct contact, e.g., HBV.
- Agent stability - survival in environment or otherwise prolonged viability (spore formation).
- Infectious dose - the dose required to cause infection in humans or animals (ID 50 refers to the dose needed to infect 50% of the exposed population).
- Antibiotic resistance.

The use of recombinant DNA may alter any of the above risk factors therefore its required that investigators take these modifications into account when working with recombinant microorganisms.

All of the above factors are inherent to a particular microbe; external factors to be considered in a risk assessment include:

- Titer/volume of material used - titer may increase several orders of magnitude compared to levels in clinical samples, upon culturing.
- Availability of effective treatment or vaccine.
- Nature of activities - e.g., potential for splashes, volume used, complexity of manipulations, skills and training level of investigators.

Health status of investigator - e.g., immune state, pregnancy, vaccination status.

Hazards of Genetically-Modified Agents

When conducting a risk assessment of genetically modified agents, consideration of the same factors used in risk assessment of the wild-type organism should be done. However, it is important to address the possibility that the genetic modification could alter (i.e., increase or decrease) the pathogenicity of the agent or affect its susceptibility to antibiotics or other treatments. Sometimes, important information may not be available for a newly engineered agent and the risk assessment may be difficult or incomplete. In these cases, due diligence should be practiced and the biosafety level assignment should be made conservatively. Once more information is available another risk assessment should be completed.

Hazards of Cell Cultures

Human and animal cells and tissues have the potential to harbor latent infectious agents and personnel who handle these materials are at risk for possible exposure. For additional information and requirements for working with human cell cultures please refer to the Exposure Control Plan and to the following section of this manual: Guidelines for Working with Tissue Culture/Cell Lines.

Laboratory Procedure Hazards

- Parenteral inoculations
  Injection of potentially hazardous materials can occur by a needle, other contaminated sharp or by bites from infected animals or arthropod vectors.
- Spills and splashes into skin and mucous membranes
  Mucous membranes include the eyes, nose and mouth.
- Ingestion through mouth pipetting
- Animal bites and scratches
- Inhalation exposures to infectious aerosols
  Aerosols, or respirable sized particles, are extremely hazardous because they are generated in many lab procedures and are usually undetected. The creation of infectious aerosols places the person carrying out the procedure and others in the laboratory at risk. Any procedure that breaks the surface tension of a liquid will produce aerosols. Pipetting, blenders, non-self contained centrifuges, sonicators and vortex mixers all produce aerosols. Procedures and equipment that create aerosols also create larger droplets that rapidly settle out of the air. These droplets can settle on surfaces and therefore contaminate gloved hands, work spaces and mucous membranes.

Agent Summary Statements

Agent Summary Statements, including recommended BSL for many laboratory microorganisms, are included in BMBL 5, Section VIII.

Other resources for biosafety hazard classification are noted on the American Biological Safety Association (ABSA) page, Risk Group Classification for Infectious Agents.

For pathogen safety data sheets visit the Public Health Agency of Canada website or contacts OEHS

Engineering Controls

Engineering controls are devices and equipment that isolate and contain a hazard. The best engineering controls function with a minimum of user input and may to a degree compensate for human error.

Biological Safety Cabinets (BSCs)

Biosafety cabinets are the primary engineering control for the minimization of exposure to potentially infectious materials. BSCs combine directional air flow and high efficiency

particulate air (HEPA) filters to protect researchers and the environment from aerosolized microorganisms. Air enters the cabinet through the face (where the investigator sits), preventing contaminants generated at the work surface from entering the laboratory. Air discharged from the cabinet first passes through a HEPA filter, removing 99.97% of particles with an aerodynamic diameter of 0.3 microns; smaller or larger particles are removed with greater efficiency. Most BSCs also protect materials used within them from contamination. All open manipulation of organisms and activities with a BSL-2 organism having potential for splashes or aerosol generation, must be performed in a BSC or similar type of containment device.

Class I BSC - Room air enters at face, circulates within the work space, and exits through the HEPA filter after passing through rear plenum. Class I cabinets closely resemble the earliest manufactured BSCs; they are infrequently used for modern research activities, because while protecting the investigator and the immediate environment, they do not protect research materials from environmental contamination. We currently don't have such BSCs at Suffolk.

Class II, type A1 BSC - Room air is drawn through the supply grille at the front of the work surface and passed through fan, entering the rear plenum. Portions of airstream pass through exhaust filter or supply filter. Only HEPA-filtered air contacts the work area, providing protection from environmental contamination of research materials. We only have class II BSC at Suffolk University.

Note: Class I and Class II, cabinets exhaust filtered air back into the laboratory. Because HEPA filters do not capture gases or vapors, volatile, toxic chemicals must not be used in these BSCs.

Procedures for Effective Use of BSCs

Appropriate user protection and contamination prevention provided by a BSC is directly related to the activities of the operator. Below are the steps to ensure that the BSC functions effectively.

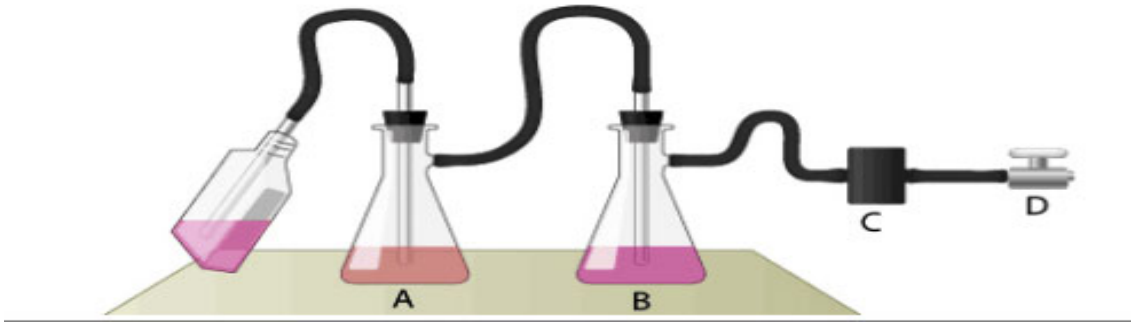
- Cabinets **shall be** certified under the following conditions:
    - Annually
    - Following relocation (including within-room). BSC on castors may be moved carefully without subsequent recertification.
    - Following HEPA filter change
    - Following service that may have affected containment ability.
    - Semi-annual certification is recommended when cabinets are used for work with airborne-transmitted organisms
    - If the airflow, indicated by magnehelic gauges fall out of an established range.
- To maintain proper directional airflow, do not block the front air intake or the rear exhaust grille and minimize the amount of material kept inside the cabinet.
- Heat from a Bunsen burner may damage HEPA filters and disrupt the protective airflow pattern. The use of disposable inoculating supplies combined with the sterile atmosphere of the BSC, should eliminate the need for heat decontamination throughout the procedure.

- Work 6 inches from the front of the cabinet, over the tray and not over the grille; avoid rapid arm movements that can disrupt airflow.
- In order to minimize arm movement in and out of the cabinet, place all needed materials in BSC at the start of procedures, arranging them to so that 'dirty' items do not pass over 'clean' ones. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials.
- Allow cabinet fan to run 5 minutes prior to and at the completion of work; wipe interior with 70% ethanol before and after work.
- Locate BSCs in low-traffic areas away from air supply grilles and doorways; drafts may disrupt protective air flow.
- Many BSCs are equipped with UV lights, but routine disinfection of work surfaces is more critical in ensuring a contaminant-free work area, and relying heavily upon the disinfection activity of the UV light is not recommended. Turn off UV lights when the cabinet is in use. UV lights should be wiped with an alcohol-moistened cloth weekly; a dust covered bulb is ineffective. Bulbs must be disposed through OEHS.
- Ensure the room door is close when working in a BSC, especially if the BSC is located near the door.
- Most BSCs have a removable work surface tray and front grille, and the space beneath it requires regular cleaning to avoid contamination problems. A schedule for regular removal of the work surface tray and disinfection of the space beneath with 10% bleach followed by 70% ethanol is recommended. The drain valve under the work surface can facilitate cleaning.

Vacuum Line HEPA Filters

Vacuum lines require periodic maintenance by University personnel and it is vital to ensure that exposures to research materials are prevented. All vacuum lines, both inside BSCs and on bench tops must be protected with a HEPA filter and a disinfectant-filled collection flask. See the following diagram for setup instructions.

The left suction flask (A) is used to collect contaminated fluids into a suitable decontamination solution; the right flask (B), serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms. Use flask(s) large enough to collect a day's worth of aspirate, nothing larger. Compression of the Tygon tubing may indicate that the filter requires replacement. Keep flasks in the BSC, not on the floor, to avoid accidental breakage or spilling. If flasks must be kept on the floor, use secondary containment such as a plastic bucket. Filters may be obtained through lab supply companies. Empty flasks daily, providing fresh disinfectant each day, to reduce the likelihood of contamination problems.



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Sharps Containers and Safe Needle Devices

Sharps Containers

Needles, razor and scalpel blades, Pasteur pipettes, serological pipettes, micropipette tips and similar items must be discarded as Regulated Medical Waste, including organisms and materials containing recombinant DNA in puncture-resistant sharps containers. Many 'sticks' and cuts are caused by improperly disposed sharp items or sharps that were left 'lying around'; keep a sharps container as close as possible to where these items are used, if possible within arm's reach.

Glass items (pipettes, test tubes) should be substituted whenever possible by plastic ones. The use of needles, blades and other sharp objects should be limited to those situations where no other alternative exists.

Safe Needle Devices

These include 'needleless systems' and sharps incorporating automatic protection features. They allow for the elimination of exposure to or automatic shielding of needles during use, minimizing the risk of 'sticks' and cuts. While mostly applicable to clinical settings, they must be incorporated whenever there is the risk of exposure to materials containing recombinant DNA, human blood, body fluids, cells, unfixed tissue or any other material covered by OSHA's Bloodborne Pathogens Standard. A copy of the BBP exposure control plan should be available in the lab when applicable.

Centrifuge Safety

Centrifuge accidents may release large volumes of infectious, aerosolized material.

- Keep accurate rotor use logs; decommission rotors as per manufacturers' recommendations.
- Inspect rotors, particularly the chambers, for corrosion and pitting.
- Use "safety cups" or covers (gasketted containers into which tubes are placed during centrifugation). If a tube breaks, the material will be contained. **An example of a centrifuge safety cup is illustrated on the next page.** These safety devices can be obtained from the manufacturer. Check with personnel in your laboratory to confirm that they have already been obtained for your lab's centrifuge(s).
- If a safety cup is unavailable, be sure the rotor cover or chamber lid is tightly closed - never use an uncovered rotor.
- For infectious materials or materials containing recombinant DNA, fill tubes and load/unload rotors or safety cups inside a BSC.

If a tube breaks during centrifugation:

- Allow aerosols to settle for 20 minutes before opening the chamber.
- Don personal protective equipment as described in Spill Procedures.
- Use a squeeze bottle to carefully apply disinfectant solution to contaminated surface, taking care to minimize splashing.
- Allow 20 minutes contact time, remove buckets and rotors to nearest BSC, aspirate residual disinfectant, and wipe down surfaces with clean water.
- Place debris in sharps containers or red bags.
- Follow manufacturers' instruction for selection of disinfectants for use on rotors and buckets. These items are usually corrosion-sensitive.
- The table below provides a list of safety equipment designed to eliminate or reduce certain hazards and briefly outlines the safety features

Examples of safety equipment use for mitigating hazards

Equipment	Hazard Corrected	Safety Features
Biological Safety Cabinet --Class I	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Does not provide product protection
--Class II (at SU)	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Provides product protection
--Class III	Aerosol and spatter	Maximum containment. Provides product protection if laminar flow air is included.
Pipetting aids	Hazards from pipetting by mouth, e.g. ingestion of pathogens, inhalation of aerosols produced by mouth suction on the pipette, blowing out of liquid or dripping from pipet, contamination of suction end of pipette	Ease of use Controls contamination of suction end of pipette, protecting pipetting aid, user, and vacuum line Can be sterilized Controls leakage from pipette tip
Loop microincinerators, disposable loops	Spatter from transfer loops	Shielded in open-ended glass or ceramic tube. Heated by gas or electricity. Disposable, no heating necessary
Leakproof vessels for collection and transport of infectious materials	Aerosols, spillage, and leakage	Leakproof construction with lid of cover Durable Autoclavable

Sharps disposal containers	Puncture wounds	Robust, puncture-proof
Transport containers between laboratories, buildings	Release of microorganisms	Robust Watertight primary and secondary containers to contain spills Absorbent materials to contain spills
Autoclaves, manual or automatic	Infectious material (made safe for disposal or reuse)	Approved design Effective heat sterilization
Screw-capped bottles	Aerosols and spillage	Effective containment
Vacuum line protection	Contamination of laboratory vacuum system with aerosols and overflow fluids	Cartridge-type filter prevents passage of aerosols (particle size 0.45 µm) Overflow flask contains appropriate disinfectant. Rubber bulb may be used to close off vacuum automatically when storage flask is full. Entire unit is autoclavable.

Work Practices

Principal Investigators are responsible for ascertaining that their staff are appropriately trained to carry out their assigned laboratory functions, but ultimately individuals bear primary responsibility for their own safety and health and therefore no one should hesitate to inquire of senior lab staff if there is an activity that you are not sure you can perform safely.

General Laboratory Practices

Routes of Exposure and Infection

An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. Through the mouth

- Eating, drinking and smoking in the laboratory
- Mouth pipetting
- Transfer of microorganisms to mouth by contaminated fingers or articles

2. Through the skin

- Accidental inoculation with a hypodermic needle, other sharp instrument or glass
- Cuts, scratches

3. Through the eye

- Splashes of infectious material into the eye
- Transfer of microorganisms to eyes by contaminated fingers

4. Through the lungs

- Inhalation of airborne microorganisms

Most of the laboratory-acquired infections reported in the literature point to accidents during work with some type of infectious agent. These include spills, splashes and accidents involving needles or other sharp objects.

Basic Precautions

- All laboratories must have a door sign that states the name, and phone number of the PI, emergency contact number(s), any entry restrictions, and for labs working at BSL-2, the universal Biohazards symbol. These signs are provided by OEHS.
- Keep laboratory doors closed when working with BSL-2 organisms.
- Drinking, chewing gum, applying cosmetics, or handling contact lenses in work areas is strictly prohibited, as is the storage of food or beverages in refrigerators/freezers used for research materials.
- Cover work surfaces with 'bench-kote' or other absorbent; use disinfectant-soaked towels for work with highly infectious material or when splashing/spattering is anticipated.
- Decontaminate work surfaces at the end of procedures and immediately after a spill. Limit bench-top items to those in immediate use; cluttered areas are more likely than well-maintained spaces to be the sites of accidents and are harder to clean and disinfect.
- After use, place reusable sharps such as surgical instruments, in puncture-resistant containers with disinfectant solution and labeled with the biohazard symbol. Detailed protocols must be developed for handling, cleaning, disinfecting and/or sterilizing any reusable sharps.
- Minimize splashing and aerosol generation. When pipetting, expel liquids against the side-wall of a tube rather than against the tube bottom. If aerosols of infectious materials will be generated, work in a BSC.
- Use secondary containers (trays, specimen transport bags) for the prolonged storage or transport of infectious materials. Whenever possible, replace glass lab ware with plastic; glass Pasteur pipettes are particularly prone to breakage.
- Never pipette by mouth.
- Use only mechanical pipetting devices and cotton-plugged pipettes; do not expel air through a pipette to mix suspensions containing infectious or toxic materials.
- Follow Good Microbiological Practices.

Fire Prevention and Biological Safety

Efforts to eliminate contamination, as practiced in some settings, run counter to basic fire prevention principals. This may occur in two types of activities:

- Dipping cover slips, cell spreaders and other items for which contamination-free status is required, into alcohol followed by flaming with a Bunsen burner. This may result in a fire when ignited alcohol drips onto flammable material.
    - Use disposable cell spreaders, which may be collected and re-sterilized by autoclaving for subsequent use.
    - Instead of dipping and flaming cover slips, autoclave batches in a glass petri dish. If kept covered in a biological safety cabinet, the slips will remain sterile.
- The use of burners in a biological safety cabinet to flame pipets, bottle and tube necks and inoculating loops needlessly places an ignition source in the work area. The environment inside the cabinet is microbiologically sterile. Using wrapped sterile pipets in this environment eliminates the need to flame. Single use inoculating loops are available and like cell spreaders may be collected and re-sterilized by

autoclaving for subsequent use. Opening and closing of tubes and bottles inside the cabinet eliminates the need for flaming the necks of these containers.

If you must use a flame or heat contact OEHS before considering purchasing a model that comes with a low-flame pilot light or a micro-incinerator that provides a heat source without an open flame.

Pipetting and Repetitive Stress Injuries

Repetitive pipetting, particularly with multi-channel devices may result in harmful stress on the arms, wrists, or shoulders. Some of this stress can be eliminated by using devices designed with ergonomic considerations. Ask your supplier about such models or contact OEHS to obtain product information. To reduce the risk of repetitive stress injuries:

- Rotate pipetting tasks among several people.
- Take short pauses of a few seconds when you can't take a longer break.
- Choose pipetters requiring the least pressure; use only the force necessary to operate it.
- Work with arms close to the body to reduce strain on shoulders.
- Keep head and shoulders in a neutral position (bent forward no more than 30 degrees).
- Use adjustable chairs or stools; high stools will force you to work with a bent neck.
- Don't elevate your arm for lengthy periods without support.

Frequent pipetting requiring thumb action to expel liquids may cause inflammation of the tendon or sheath used for this motion; use pipettes that expel liquids with a "clenching-the-fist" motion.

Personal Protective Equipment

The appropriate use of personnel protective equipment (PPE) is critical in reducing exposure to potentially infectious materials. PPEs are considered as the 'last line of defense' when risk assessment does not indicate that engineering controls and work practices can be relied upon for adequate protection. These situations frequently exist, necessitating the use of PPE. Also ensure that PPEs are used in the proper context.

Gloves

Gloves must be worn whenever handling infectious materials. Users of latex gloves are at risk for developing allergies to latex. Nitrile or vinyl gloves should be used instead of latex. Those who prefer latex should use only powder-free gloves that are designated "low protein" by the manufacturer. Glove manufacturers are able provide documentation for their products' resistance to permeation as it is prudent to be aware of when you need to review that information.

Corrosives and organic solvents may penetrate gloves or diminish their protective ability; it may be necessary to stock more than one type of glove for the full range of a laboratory's activities. Glove compatibility information is available from glove manufacturers, or consult the OEHS.

When using any glove:

- Check for visible tears and other defects.
- Remove rings and other jewelry if they may rip gloves.
- Protective ability diminishes as gloves are worn due to stretching and abrasion; change gloves regularly or as soon as possible if they are overtly contaminated.
- Wash hands immediately after removing gloves.
- Remove gloves when leaving the laboratory; even if they are "clean", their presence in an elevator or other common area justifiably causes misgivings among other building occupants - they do not want to turn the same door knob. (Its required to properly decontaminate the exterior surfaces of containers used to transport infectious materials and eliminate the perceived need to wear gloves during transport on campus.)

Eye Protection

Eye injuries are among the most preventable types of laboratory accidents. Glasses routinely worn for vision correction do not provide the appropriate level of protection for work with hazardous materials.

- Safety glasses with side shields provide the minimum level of protection for handling any hazardous material.
- Goggles, which unlike safety glasses fit tightly all around the eyes are required for activities with a small splash hazard or work with organisms transmissible through mucous membrane exposure.
- Goggles are used with a face shield when an elevated risk of large quantity splashes exists or when working with highly toxic, corrosive, or infectious materials.
- Face shields must also be used for protection against UV radiation (be sure that the face shield carries the manufacturer’s validation of UV protection) and when handling liquid nitrogen.

Lab Coats

Lab coats must not be worn outside of the laboratory if they were used during work with infectious materials. Wear coats resistant to liquid penetration for activities with splash potential or use a plasticized apron. For high risk activities, use a rear-fastening lab coat. Provision, laundering, and replacement of lab coats is the responsibility of the Principal Investigator, or Department; employees are not allowed to launder contaminated lab coats at their home.

Surgical Masks

Masks will help prevent ingestion and protect the mucous membranes of the nose and mouth. They **do not** provide sufficient protection against infection from organisms transmitted by inhalation.

Respirators

Respirators are used when there is the risk of airborne exposure to organisms transmitted by inhalation and containment devices are unavailable or unable to provide sufficient protection. Respirators use must be preceded by medical clearance, training, and fit testing. Follow the university respiratory protection program. These services must be arranged through OEHS.

Laboratory Equipment

Laboratory equipment use poses related mechanical, electrical energy and the materials used in them. Some equipment may be one-of-a-kind with each requiring its own learning curve before it can be safely used. Some equipment becomes obsolete relatively quickly and with each new piece comes the requirement to relearn its operation.

Be sure that 'owners' manuals' are readily accessible and when in doubt, contact a customer service representative. Do-it-yourself fixes are not only dangerous but may **invalidate warranties**. Senior lab personnel should be responsible for ensuring that new staff are familiar with the safe operation of equipment.

There also may be specific requirements for moving sophisticating machinery in which case a customer service official should be contacted or the users' manual carefully reviewed.

Water Baths

Water baths may become contaminated by organisms incubated in them or through amplification of water or airborne organisms. Iodine-based or phenolic disinfectants are recommended for intermediate temperature baths. A 1/1,000 dilution of household bleach is also effective but may corrode water bath components. It has been reported that placing a few pennies (copper) in the bath will inhibit microbial growth.

- Never use sodium azide; it is fairly toxic and drain disposal is illegal and may result in the formation of explosive metal azides. Consult the manufacturer to determine the recommended disinfectant.
- Do not leave water baths on overnight or when they will be unattended for extended periods**.**

Cryostats

Cryostats should be regularly decontaminated with appropriate hospital grade disinfectant. Trimmings and tissue sections should be treated as potentially infectious. Never attempt to clear debris from a blade with your finger; always use a brush or other mechanical device to prevent contact with the blade. When changing blades use protective gloves and handle the blades with forceps or tongs. Pre-soaking blades in a disinfectant solution prior to cleaning (removal of debris) will reduce the number of viable microorganisms.

Mixers, Sonicators, and Blenders

Mixers, sonicators, and blenders produce large quantities of aerosols. Models designed to contain aerosols are available. These devices should be operated within a BSC with a disinfectant-moistened towel placed over the top. Open only after allowing sufficient time for aerosols to settle. If possible, avoid using glass bowls. Sonication may be safely performed by placing a tightly capped specimen tube in a beaker of water and putting the probe in the **water**, not in the tube.

Needles and Syringes

Sharps should only be used when no other alternative is available and especially in BSL 2 or above labs.

- Use blunt needles, pipettes, or canulas to aspirate fluids instead of hypodermic needles; substitute plastic for glass when possible.
- Use only needle-locking units or units in which the needle is an integral part of the syringe.
- Dispose all needles properly in a "sharps" container immediately after use.
- Dispose of unused needles in sharps containers.
- Never recap, shear, break, or bend needles under any circumstances. Expel air and bubbles into a disinfectant-moistened pad.
- Review information on safer needle devices.

Lyophilizers

Lyophilizers produce a dry solid that is very easily dispersed. They should be fitted with a HEPA filter or vented to a BSC when used for drying suspensions of infectious material. Ampoules of lyophilized solids should be opened only in a BSC; place a disinfectant-moistened pad over the scored line when opening the ampoule. Disinfect chamber surfaces and any material collected in the vapor trap.

Decontamination

Decontamination refers to any activity that reduces the microbial load to a level deemed suitable to prevent contamination or infection. The appropriateness of a decontamination procedure is situation-dependent

Antisepsis refers to the application of a chemical to living tissue to prevent infection. Examples include iodine compounds and antimicrobial soaps for hand washing.

Sterilization

Sterilization refers to the destruction of all microbial life, including bacterial endospores.

Autoclaves - Autoclaves provide the most efficient and reliable method of sterilization for most laboratory applications. The critical process factors are temperature, exposure time, and ensuring that materials are packaged to allow the steam to penetrate throughout the load. Sterilization time will vary in relation to the size of the load and the packing density of the chamber. Typical laboratory autoclaves operate at 121°C and 15 psi. All users must review the operating manual periodically. Instructions should be prominently posted.

Use heat resistant gloves and face protection, particularly when removing processed material; crack the door slowly and wait a few minutes before fully opening it.

For dry loads, add 250-500 ml. of water to the load pan to aid in steam generation. Autoclave bags should be closed loosely to allow steam to penetrate; do not tightly cap bottles and test tubes.

Autoclave tape is not a fail-safe indicator of sterilization; it blackens after only brief exposure to a temperature of 121°C. When used for sterilizing infectious waste, autoclave performance must be periodically validated by using *B. stearothermophilus* spore vials. Place a vial in a hard-to-reach area of a mock challenge load and attach a string to facilitate removal after autoclaving. Incubate as directed; a lack of turbidity indicates that the autoclave is achieving sterilizing conditions.

Some autoclave tapes contain lead which makes it necessary to dispose of these tapes as Hazardous Waste. Laboratories must use lead-free autoclave tape to eliminate this hazardous waste stream. Check with your lab supplies vendor for the lead-free autoclave tape options or conduct OEHS for assistance.

Dry Heat - Dry heat is used for materials (some glassware, instruments, and anhydrous materials) that are sensitive to moisture or the corrosion it may cause. Consult the manufacturers of such items for recommendations for appropriate sterilization procedures. Dry heat requires higher temperatures and a longer exposure times than autoclaving. Dry heat for 2-4 hours at 160°C is needed to sterilize a load requiring 30 minutes at 121°C in an autoclave. This method may also be validated by using spore vials; see autoclave section (above).

Chemical Sterilization - Chemical sterilization is chiefly used for heat-sensitive patient-care instruments that enter body cavities or normally sterile areas. This process requires prolonged contact times with relatively highly concentrated solutions. Some sterilants require that specific ventilation systems be in place to remove hazardous gases and vapors.These method is not recommended at SU and if you are considering it consult with OEHS if you must consider this option.

Disinfection

Disinfection refers to the elimination of virtually all pathogenic microorganisms on inanimate objects with the exception of large numbers of bacterial endospores.

Disinfection encompasses a continuum of outcomes in terms of the types of organisms destroyed. Microorganisms can be grouped in terms of decreasing resistance to disinfectants as follows: bacterial endospores (*B. subtilis, clostridium spp*); Mycobacteria; nonlipid or small viruses (poliovirus, rhinovirus); fungi; vegetative bacteria; and lipid or medium sized virus (herpes simplex, HIV, HBV).

Review the framework for the selection of the appropriate disinfectant. The lists of EPA registered disinfectants can be found at http://www.epa.gov/oppad001/chemregindex.htm

The EPA does not independently audit such results and research indicates that in real life situations some products do not perform as claimed. This result from manufacturers testing their products in best-case situations, e.g., on a smooth surface, at an optimal pH, in a buffer solution instead of a solution containing organic material which partially inactivates some disinfectants. For high risk pathogens, investigators may devise their own test that best represents their circumstances to confirm a product's claim.

When using any disinfectant:

- Follow label instructions for dilution and contact time needed for desired level of disinfection.
- Disinfectants that require pre-use dilution should be treated as hazardous chemicals during mixing. Wear a lab coat, the correct type of chemical-resistant glove, and goggles, not glasses.
- Clean contaminated surfaces as soon as possible and any surface that may have become contaminated at the end of the task.
- Select the disinfectant with the lowest toxicity possible

Considerations for selecting and using disinfectants:

- Nature of surface-rough surfaces require a longer contact time than smooth ones.
- Surface compatibility-bleach will corrode many metals, rinse with water after use; instruments vary in their ability to withstand disinfectants based on their composition.
- Organic matter will inactivate some disinfectants; a second application may be necessary once visible contamination (and hence, most organic debris) has been removed. The removal of visible 'soil' may be the single most critical factor in assuring effective decontamination.
- Resistance of microorganisms, e.g. bacterial endospore vs. vegetative bacteria.
- Number of microorganisms present, overnight culture vs. a recently inoculated one.

The Bloodborne Pathogens Standard requires that products labeled "tuberculocidal hospital disinfectant" be used on surfaces and equipment when the Standard is in force. Household bleach, usually at a 1/10 dilution, also satisfies this requirement and may be used in these

cases. Bleach solutions should be prepared fresh daily because they lose potency over time.

Summary of Disinfectant Activities

Disinfectant	Disinfection Level	Bacteria	Lipophil. Viruses	Hydro-Philic Viruses	M. tuber-culosis	Fungi	Comments
Alcohols (ethyl and isopropyl) 60-85%	intermediate	+	+	-	+/-	+	Not sporicidal; evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable.
Phenolics (0.4%-5%)	intermediate	+	+	+/-	+	+	Not sporicidal; phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue.
Glutaraldehyde (2-5%)	high	+	+	+	+	+	Used to sterilize surgical instruments that cannot be autoclaved; strong odor; sensitizer; use with adequate ventilation. Not for use on Environmental surfaces.
Quaternary Ammonium (0.5-1.5%)	low	+	+	-	-	+/-	May be ineffective against Pseudomonas and other gram – bacteria; recommendation limited to Environmental sanitation (floors, walls). Low odor, irritation.
Iodophors (30-1,000 ppm iodine)	intermediate	+	+	+	+/-	+/-	Inactivated by organic matter.
Chlorine (100-1,000 ppm)	intermediate	+	+	+	+/-	+	Not sporicidal; inactivated by organic matter; fresh solutions of hypochlorite (chlorox) should be prepared daily; corrosive; irritating to eyes and skin.

Alcohols:

Ethyl or isopropyl alcohol in concentration of 70% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores.

Formalin:

Formalin is 37% solution of formaldehyde in water. Dilution of formalin to 5% results in an effective disinfectant. Formaldehyde is a suspected human carcinogen and creates respiratory problems at low levels of concentration.

Glutaraldehyde:

This compound although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehydes are irritating to the eyes, nasal passages and upper respiratory tract. They should always be used in accordance with the instructions on the label and the appropriate personal protective equipment.

Phenol and Phenol Derivatives:

Phenol based disinfectants come in various concentrations ranging primarily from 5% to 10 %. These derivatives, including phenol, have an odor which can be somewhat unpleasant. Phenol itself is toxic and appropriate personal protective equipment is necessary during application. The phenolic disinfectants are used frequently for disinfection of contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including Mycobacterium tuberculosis, fungi and lipid-containing viruses. They are not active against spores or non-lipid viruses.

Quaternary Ammonium Compounds (Quats):

Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. They are less active against Gram-negative bacteria and are not active against non-lipid-containing viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general cleaning.

Halogens (Chlorine and Iodine):

Chlorine-containing solutions have broad spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Chlorine containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilants since they inactivate bacterial spores. Iodine has similar properties to chlorine. Iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

Using bleach as a disinfectant

The sodium hypochlorite in household bleach is a strong oxidizing agent that is an effective disinfectant for the known, and potential, infectious materials at Suffolk. However, over time the sodium hypochlorite breaks down to salt and water.
When bleach and water are mixed together, 1:10, to create a cleaning or disinfecting solution, the solution rapidly begins to lose needed disinfecting properties. Hence the recommendation to for the solution to be made fresh daily.

Stock bleach should be stored in an opaque plastic bottle at room temperature. The rate of degradation depends on the initial hypochlorite concentration, the ambient temperature and the volume remaining. Manufacturers are not required to put an expiration date on the bottle. A good practice is to mark the bottle with the receive date, and replace bleach that was received more than 6 months prior. Colorimetric test strips for hypochlorite concentration provide an easy and useful monitoring tool.

The potency of commercial bleach is between 3.25 and 6.15% hypochlorite, depending on manufacturer, they are not created equal. Ultra regular CLOROX liquid bleach contains a higher concentration.

Gloves should be worn while handling bleach. Bleach can be corrosive on some surfaces, including steel. Bleach residue on non-porous surfaces should be wiped off with water or 70% ethanol. Bleach should not be used in conjunction with other household cleaning products that contain ammonia; the two can react to produce a highly toxic product. Pre-filled spray bottles that mix at the nozzle are a convenient way of generating a 1:10 mixture for use in the lab.

Aspiration of tissue culture media into a collection flask, under vacuum, is one of the most commonly performed laboratory procedures. University Policy requires that such media be decontaminated prior to disposal in the municipal sewer system.

Effective decontamination is simple, following these instructions. Before aspiration, add undiluted bleach to fill 10% of the final volume of the collection flask. Bleach is an effective decontaminant with the added advantage that its strong oxidizing properties will turn the phenol red indicator in tissue culture media from pink to yellow/clear. Aspiration flasks containing pink liquid indicate insufficient bleach concentration, and should be topped off with fresh bleach until a yellow/clear color is achieved prior to additional aspiration or disposal. Empty the collection flasks when they are 3/4 full, or at least weekly.

Spill Procedures

Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards should have a basic biological spill kit ready to use at all times. For most instances the basic kit can be assembled with materials already used in the laboratory. All labs operating at BSL-2 must have an assembled spill kit available in the lab. In BSL-1 labs materials should be easily accessible to everyone in the lab, prior assembly might not be necessary. Ask for assistance from OEHS regarding spill kits or their assembly.

The following is a list of items that should go into a basic biological spill kit. It should be enhanced to meet the needs of your unique situation.

- Disinfectant (e.g., bleach 1:10 dilution, prepared fresh)
- Absorbent material (e.g., paper towels, absorbent powder)
- Waste container (e.g., biohazard bags, sharps containers)
- Personal protective equipment (e.g., gloves, eye and face protection)
- Mechanical tools (e.g., tongs, dustpan and broom)
- Antimicrobial towelettes
- Spill cleanup procedures

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency situation, stay calm, leave the area if needed and call SUPD X8333 and proceed with common sense. SUPD dispatch will notify OEHS immediately.

Spills inside the Laboratory

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further. Have a complete biological spill kit ready to go before you start the clean-up.

Spills with NO broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Cover the spill area with absorbent material (i.e., Superfine or paper towels).
5. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag, or if using paper towels, place them in the biohazard bag for disposal.
6. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
7. Repeat step 6 to allow for sufficient disinfection of contaminated surfaces.
8. Remove outer pair of gloves only and dispose of them in the biohazard bag.
9. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
10. Remove inner gloves and dispose of them in biohazard bag.
11. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
12. Wash your hands with soap and water as soon as possible.
13. Restock the kit for next use.

Spills inside the Laboratory

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further processing by laundry (SU or department). Have a complete biological spill kit ready to go before you start the clean-up.

Spills with NO broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Cover the spill area with absorbent material (i.e., Superfine or paper towels).
5. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag, or if using paper towels, place them in the biohazard bag for disposal.
6. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
7. Repeat step 6 to allow for sufficient disinfection of contaminated surfaces.
8. Remove outer pair of gloves only and dispose of them in the biohazard bag.
9. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
10. Remove inner gloves and dispose of them in biohazard bag.
11. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
12. Wash your hands with soap and water as soon as possible.
13. Restock the kit for next use.

Spills inside the Laboratory

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further processing by laundry (SU or department). Have a complete biological spill kit ready to go before you start the clean-up.

Spills involving broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Using tongs or forceps, place broken glass/sharps in sharps container.
5. Cover the spill area with absorbent powder.
6. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag.
7. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
8. Repeat step 7 to allow for sufficient disinfection of contaminated surfaces.
9. Remove outer pair of gloves only and dispose of them in the biohazard bag.
10. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
11. Remove inner gloves and dispose of them in biohazard bag.
12. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
13. Wash your hands with soap and water as soon as possible.
14. Restock the kit for next use.

Spills inside the Biological Safety Cabinet

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

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

Spills Inside a Centrifuge

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

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

Spills during Transport

If a spill occurs in a public area:

- Don't attempt cleanup without the proper supplies.
- Notify SUPD (X8333) immediately and they will contact OEHS.

Spill kit maintenance:

Your biological spill kit should be restocked after each use. It should also be checked for completeness on an annual basis. The following maintenance activities should be done:

- Check expiration on disinfectant and replace as needed (e.g., bleach should be replaced annually);
- Replace gloves;
- Replace antimicrobial towelettes; and
- Check straps on splash goggles for deterioration.

Handwashing and Hand Decontamination

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 20 seconds, rinsed in clean water and dried.

Foot- or elbow-operated faucets are recommended. Where not available, a paper towel should be used to turn off the faucet handles to avoid re-contaminating washed hands.

Alcohol-based hand-rubs may be used to decontaminate lightly soiled hand when proper hand-washing is not available. The use of hand-rubs should be followed up with a soap and water

wash as soon as possible.

Tissue Cultures and Cell Lines

Cell lines obtained from commercial sources may become contaminated with adventitious agents while used in the laboratory. The extent of screening varies among providers and while most test for bacteria, mycoplasma, and fungi, they do not routinely include testing for viruses other than those categorized as 'Bloodborne Pathogens'.

Cell cultures known to contain an infectious agent or oncogenic virus should be manipulated at the Biosafety Level appropriate for the agent, usually BSL-2 is the minimum.

For activities with materials not known to contain infectious agents, the following hazard classification applies:

BSL-1 is appropriate for well-established lines of cells of sub-primate origin if they do not harbor a primate virus and are free of bacteria, fungi, and mycoplasma. However, working with these materials at BSL-2 is recommended because of the additional degree of protection from contamination provided by BSL-2 practices, particularly the use of a Biological Safety Cabinet.

BSL-2 is appropriate for activities with: all primate cell lines, even well established ones, all cells derived from primate lymphoid or tumor tissues; all primate tissue; all human clinical material*; cultured cells new to the laboratory until proven contaminant-free; and, cells exposed to or transformed by a primate oncogenic virus.

*These activities and the use of any cells purposely infected with or suspected of harboring agents defined as bloodborne pathogens are covered by the OSHA Bloodborne Pathogens Standard. Laboratories using human cell strains (non-transformed cells) propagated from primary explants must also comply with the Standard because they are considered "unfixed human tissue" which is covered by the regulation.

Biosecurity

Recent events have brought to the forefront the necessity of having a comprehensive laboratory security program. However, before outlining the biosecurity requirements that have been implemented by the University it is important to understand the distinction between "biosafety" and "biosecurity."

"Biosafety" is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or other biohazards. "Biosecurity" refers to measures designed to protect microbiological agents from loss, theft, misuse or intentional release, and to protect research-related information from loss, theft or misuse. This can be accomplished by limiting access to facilities, biological materials and research-related information. Sufficient security for the biological materials in use may already be in place for laboratories that do not handle select agents, exempt levels of toxins on the select agents list or exempt strains of select agents. These security measures include access controls and training requirements outlined for BSL-1 and BSL-2 laboratories previously.

Elements of the biosecurity program include:

1. **Physical security:** Access control and monitoring are intended to prevent the removal of materials for unauthorized purposes. Access should be limited to authorized personnel based on the necessity of entering sensitive areas. At a minimum, laboratory doors must be locked when no one is present in the lab, all storage units housed in shared space (i.e., hallway, storage room, etc.) must be locked, and all persons entering the laboratory should be asked for identification and questioned as to their purpose for being there.

2. **Inventory and accountability:** It is the responsibility of each laboratory to establish material accountability procedures. These should be designed to track the inventory, storage, use, transfer and destruction of biological materials. The purpose is to know what agents are housed in a lab, where they are located and if they are all accounted for.

3. **Transport of biological agents:** Material transport policies are in place that outline requirements for transporting locally on campus and outside of campus..

4. **Reporting and communication:** In addition to following departmental reporting requirements should a security breach occur, the laboratory must also notify SUPD and OEHS. Investigation into the breach will occur as appropriate.

5. **Training:** Laboratory security awareness training is briefly addressed for anyone who has access to a laboratory. This training is available through our lab safety training, Bloodborne Pathogens Initial, as well as the refresher trainings. However, if a PI or department request for an extended training OEHS can provide one.

Hazardous Materials: Registration and Approval

Recombinant DNA

Recombinant DNA refers to either: (i) molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecule that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

The NIH's Guidelines for Research Involving Recombinant DNA Molecules apply to **all rDNA activities** at Suffolk University, regardless of the funding source for a particular project.
The NIH's risk assessment criteria for most viral vectors give very little weight to 'replication deficiency' alone. This requires applying the same hazard assumptions as if wild type virus were being used, as well as commensurate biological safety procedures.

All uses of rDNA must be described in detail and filed with relevant university safety committees and OEHS. The NIH defines protocols which are 'exempt' from submission requirements, but this category is narrower than most people assume and investigators must, at a minimum, verify and

document this determination.

Other Hazardous Materials Requiring Registration and Approval

The intended use of Infectious Agents , use of human materials (blood, body fluids, tissues, cells, human cell lines, or use of hazardous chemicals or biological toxins must also be documented and filed with OEHS and other relevant university committees/offices.

Regulated Medical Waste

Regulated Medical Waste (RMW) Regulated Medical Waste (RMW) is material that may be contaminated with blood, bodily fluids, or other infectious materials, as well as sharps. RMW must be properly handled, collected, segregated, packaged, stored, labeled, transported and disposed of in order to minimize the risk of transmitting infection or endangering human health.

Containers for Regulated Medical Waste

Contact OEHS for assistance

Sharps Containers are for disposal of items contaminated with infectious materials or recombinant DNA that may rip or poke a hole in a red plastic bag, including:

1. All hypodermic needles, suture needles, syringes, and scalpel blades, even if unused.
2. Pasteur pipettes (glass or plastic) blood vials, razor blades, serological pipettes (glass or plastic), slides, cover slips, and glass culture dishes and test tubes containing or that were in contact with cultures/stocks of microorganisms, or if they are unwrapped/unpackaged or appear as anything other than unused.
3. Devices and materials listed in the bullet above, may be placed in cardboard boxes (see below) if they are unused and in their original packaging.
4. Broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.

Red bags are for items that are not expected to poke or tear the bag when it is lifted. This includes all of following if they are unwrapped/unpackaged or appear as anything other than unused: plastic test tubes, eppendorf tubes, plastic culture dishes, gloves, tissue culture flasks. In addition, the following materials belong in red bag disposal:

- Cultures and stocks of infectious agents and associated biological materials, including cultures from medical and pathological laboratories, cultures and stocks of infectious agents from research laboratories, wastes from the production of biological materials, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate, and mix cultures.
- Human pathological wastes, including tissues, organs, and body parts and body fluids that removed during surgery or autopsy, or other medical procedures, and specimens of body fluids and their containers.

- Liquid waste, human blood, products of human blood, items saturated and/or dripping with human blood, or items that were saturated and/or dripping with human blood that are now caked with dried human blood including serum, plasma, and other blood components, and their containers, which were used or intended for use in either patient care, testing and laboratory analysis or the development of pharmaceuticals. Intravenous bags are also included in this category.
- Contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research (including research in veterinary hospitals), production of biologicals, or testing of pharmaceuticals.
- Wastes from surgery or autopsy that were in contact with infectious agents including soiled dressings, sponges, drapes, lavage tubes, drainage sets, underpads and surgical gloves.
- Laboratory wastes from medical, pathological, pharmaceutical, or other research, commercial, or industrial laboratories that were in contact with infectious agents, including disposable gloves, laboratory coats and aprons.
- Dialysis wastes that were in contact with the blood of patients undergoing hemodialysis or renal dialysis, including contaminated disposable equipment and supplies such as tubing, filters, disposable sheets, towels, gloves, aprons, and laboratory coats.
- Biological waste and discarded materials contaminated with blood, excretion, exudates, or secretion from humans who are isolated to protect others from certain highly communicable diseases, or isolated animals known to be infected with highly communicable diseases.

Cardboard and Styrofoam Boxes - Cardboard boxes should be used for disposal of the following uncontaminated, breakable items: glass labware, wrapped pipettes, slides, petri dishes and cover slips in their original packages, staining dishes, etc. These are not RMW. In some cases styrofoam boxes may be returned by the lab to the manufacturer for recycling and/or reuse.

Tissues Fixed in a Hazardous Chemical (e.g., formaldehyde, formalin). If tubes/jars containing small amounts of tissue are being discarded (e.g. mouse and rat organs), the liquid fixative must be decanted/strained off and treated as a hazardous waste. The tissue can be deposited into a red regulated medical waste bag. If jars containing large quantities of fixed tissue are being discarded (e.g. pig or human organs), please contact OEHS for a consultation. Animal carcasses that have been perfused and contain no free fixative do not need to be treated differently from unfixed carcasses.

Obtaining and Discarding Containers - All laboratories are responsible for safely and securely packaging their Regulated Medical Waste in accordance with all applicable regulations and campus- or building-specific procedures. RMW and sharps must be packaged appropriately and moved to the main waste accumulation area for offsite shipping. OEHS provides the hazardous waste management training and facilities all hazardous waste removal from the university.

Bloodborne Pathogens

The OSHA Bloodborne Pathogens Standard covers all employees with "reasonably anticipated" exposure to human blood, blood products, or other material capable of transmitting HIV, HBV, HCV and other bloodborne diseases. The law requires that employers develop and implement an Exposure Control Plan that:

- Identifies job titles and tasks where exposure may occur.
- Describes the procedures that will be used to minimize exposure risk:
- Working at BSL-2 with emphasis on engineering controls as the preferred type of control measure
- Adopting Universal Precautions-treating all human blood, certain body fluids, and other materials as if they were known to be infectious for bloodborne diseases.
- Details procedures to ensure rapid follow-up treatment consistent with current medical recommendations for employees, paid for by the employer, in the event of an exposure incident.
- Offers affected employees the HBV vaccine free of charge and the option of accepting it at any later date if the initial offer is declined.
- Provides a schedule for the regular cleaning and decontamination of work surfaces.
- Provides employees with initial and annual retraining focused on work practices that will minimize their risk of exposure.

OEHS conducts mandatory safety training sessions in a classroom setting on regular basis and be reached to schedule for training. Personnel receiving training for the first time must attend a live training session.

A hard copy of the required Exposure Control Plan is available from OEHS

PIs can modify the plan with site (laboratory)-specific information but consult OEHS first.

Biological Safety Training

Training Requirements

Lab Safety Training

All lab personnel must attend mandatory annual lab safety training with annual refreshers provided by OEHS.

Bloodborne Pathogens/Biological Safety

All University personnel working with: human blood/body fluids/unfixed tissue, human or non-human primate cell lines or any materials deemed capable of transmitting HIV, HBV, HCV or other bloodborne diseases; infectious microorganisms classified at Biosafety Level-2 (agents capable of causing disease in healthy adults); or viral vectors classified by the NIH as requiring Biosafety Level-2 procedures. Refresher training is required annually.

Biological Materials Shipping Guidelines

Shipments of certain biological materials are regulated domestically by the Department of Transportation (DOT) as well as the Federal Aviation Administration (FAA), and the International Air Transportation Association (IATA), if sent internationally. Any university member involved in packaging materials, preparing samples for shipping, handling such packages, preparing related paperwork, or signing to authorize shipments must undergo specific training. OEHS can coordinate access to this training.

There are 4 basic classifications for shipments of biological materials:

- Non-regulated biological material
- Exempt Human or Animal Specimens
- Biological Substance, Category B
- Biological Substance, Category A

Additional information on classification is provided in this **DOT brochure** (http://www.phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/Transporting_Infectious_Substances_brochure.pdf)

OEHS is available for technical consultation throughout this process regardless of shipment type or class.

Use of Human Subjects and Materials in Research

Federal and University regulations and policies require that all research involving human subjects or materials be reviewed and approved before initiation by the University's Institutional Review Board (IRB) to protect the rights and welfare of human subjects.

Suffolk University IRB is the Human Research Protection Program. Prescribed by the National Research Act of 1974 (PL 93-348) and endorsed by the Academic Council, the IRB reviews applications for research involving human subjects. Reviews are performed in accordance with the U.S. Department of Health and Human Services (HHS) regulations for the Protection of Human Research Subjects (45 CFR 46, as amended) as codified and FDA regulations and guidelines.

It is the responsibility of the Project Investigator to assure that all research involving human subjects is reviewed and approved by the IRB prior to initiation. All personnel with a reasonable anticipated risk of exposure to bloodborne pathogens through the contact with human blood or other human materials must be included in SU's Bloodborne Pathogen Program.

For more information, contact the IRB office or you can start here using the decision charts.

Suffolk University offers the Collaborative Institutional Training Initiative (CITI) program as its human subjects research ethics training curriculum. All individuals working directly with human subjects or data that can be linked back to individual subjects must complete human subjects research training requirements every two years. Research protocols will not be reviewed prior to the completion of training by all study team personnel. To complete CITI you must first register by visiting the training log-in webpage,

Appendix A: Biohazardous Material Registration Form

Appendix B: Laboratory Specific Biosafety guidelines and SOPs (BLS 2 O' Seaghdha Laboratory)

Appendix C: Biological Safety Risk Assessment Form

Appendix D: BSL – 2 Lab inspection checklist

Appendix E: Exposure Control Plan

Appendix F: Hazardous Waste Management Manual

Appendix G: Current approved research Protocols

Appendix H: Important Shared Equipment SOPs

Appendix I: Agent Summary Statements

References

OSHA Bloodborne Pathogen Standard
OSHA Laboratory Standard
BMBL 5
NIH rDNA Guidelines
SU Hazard Communication Plan
SU Chemical Hygiene Plan
SU Respiratory Protection Program
SU – IRB Website
Tufts University – Biosafety Manual
Columbia – University Biosafety Manual
Ohio State – Biosafety Manual

Appendix C:

Biological Safety Risk Assessment for Proposed Procedures

Date:_____ Principal Investigator:_____

Description of Materials & Procedures:_____

This form consists of 3 sections. Please complete this form in conjunction with the OEHS.

SECTION 1

Material Source Information

Use this space to identify:

- Types of materials to be used including quantities and biological activation status
- Source, and any known infectious disease considerations associated with either the source species or the geographic location of the source species
- Procedural steps for the analysis, from material preparation through waste disposal

SECTION 2 **Infectious Disease Considerations** Complete this section for each agent identified as an infectious disease consideration in the previous section. Make additional copies of this section if needed.		
Agent		
Pathogenicity of the organism & Routes of transmission	Infectious Dose	
	Routes of Transmission	
	Host Range	
	Disease Severity	
	Previous History of Lab-Associated Infection	
Medical Surveillance	Pre-exposure recommendations (vaccines availability, indications, etc.)	
	Post-exposure recommendations (therapy or post-exposure prophylaxis availability, indications, etc.)	
	Personnel considerations (identify any health status conditions that would make a person more susceptible to infection or for who exposure to this agent is contraindicated.)	
Agent Stability & Specific Features	Means of chemical or physical inactivation	
	Any specific qualities of the agent that will hinder inactivation or medical treatment (i.e. antibiotic-resistance, genetic modification, etc.	

Biosafety Level & Containment Practices Assignment ***(Consult with the Biosafety Office as needed))***

Use this space to summarize:

- Regulatory recommendation or restriction factors (USDA, CDC, etc.)
- Factors associated with the process that impact biosafety level assignment
- Biosafety level assignment along with any additional procedural considerations

Date of implementation: Date due for review:

Note that any biological exposure incident associated with the outlined procedure may be indicative of a need for procedural change. In this instance, a review of the procedure and the risk assessment document must be conducted within 30 days of a biological exposure incident.

Appendix D: BSL – 2 Lab inspection checklist