KGI KECK GRADUATE INSTITUTE

Biological Safety Manual

Revised by Jasmine Yu on July 30, 2024

Reviewed and Approved by LCABS committee on March 30, 2020

KGI Safety Committees

Campus Safety		Extension
Campus Security		78736
Facility Helpline		70144

Laboratory, Chemical and Biological Safety (LCABS) Committee		Extension
Loren Martin	Associate Vice Provost of Research (Committee Chair)	70388
Jasmine Yu	Laboratory Safety Manager	78698
Trevor Garrett	Assistant Vice President, Financial planning & Analysis	70002
Anastasia Levitin	Faculty	70154
John Krstenansky	Faculty	70158

Facilities Safety		Extension
Gabriela Toledo	Facilities Manager	78722
Trevor Garrett	Assistant Vice President, Financial Planning & Analysis	70002

Biological Safety Manual

Contents

A. Foreword

The biosafety manual for Keck Graduate Institute has been adopted to accomplish the following goals:

- Protect personnel from exposure to infectious agents
- Prevent environmental contamination
- Provide an environment for high quality research while maintaining a safe work place
- Comply with applicable federal, state and local requirements

The biosafety manual provides university-wide safety guidelines, policies, and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI), its success depends largely on the combined efforts of laboratory supervisors, managers, and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used. In general, the handling and manipulation of biological agents and toxins, as well as recombinant DNA molecules, requires the use of various precautionary measures depending on the materials involved. This manual will provide assistance in the evaluation, containment, and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary.

B. Biological Safety and Biosafety Levels

Biological safety (or biosafety) is the application of knowledge, techniques, and equipment to prevent personal, laboratory, and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. It can be accomplished through the following means:

Primary Containment

Protection of personnel and the immediate laboratory environment through **good microbiological technique** (laboratory practice) and the use of **appropriate safety equipment** such as a biosafety cabinet.

Secondary Containment

Protection of the environment external to the laboratory from exposure to infectious materials through a combination of **facility design** and **operational practices**.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment.

Currently four Biosafety Levels (1-4) define the level of containment necessary to protect personnel and the environment. A Biosafety Level 1 (BL-1) is the least restrictive, while Biosafety Level 4 (BL-4) requires a special containment laboratory or facility, which is not available at KGI. Since most of the research at KGI is conducted at Biosafety Levels 1 and 2, this manual will mainly focus on these two Biosafety Levels. For more information on Biosafety Levels 3 and 4 requirements, refer to the appropriate literature or contact the Biological Safety Officer.

The most important element in maintaining a safe work environment is **strict adherence to good microbiological and laboratory practices and techniques**. Everybody working with infectious agents or potentially infected materials must be aware of the potential risks. In addition, they must be trained and proficient in the practices and techniques required for handling such material. **It is the responsibility of the Principal Investigator or person in charge of the laboratory to provide or arrange for appropriate training of all personnel**.

Table 1. Summary of Biosafety Levels for Infectious Agents (BL-1 to BL-2)

Biosafety Level 1 (BL-1)	
Agents:	Not known to cause disease in healthy adults
Practices:	Standard microbiological practices
Safety Equipment: (Primary Barriers)	None required
Facilities: (Secondary Barriers)	Open bench top sink required

Biosafety Level 2 (BL-2)	
Agents:	Associated with human disease, hazard (exposure) = auto-inoculation, ingestion, mucous membrane exposure
Practices:	BL-1 practice plus: Limited access; biohazard warning signs; "Sharps" precautions; biosafety manual defining any needed waste decontamination or medical surveillance policies
Safety Equipment: (Primary Barriers)	Primary barriers = Class I or II Biological Safety Cabinets (BSCs) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; Personal Protective Equipment (PPE): laboratory coats, gloves, face and eye protection as needed
Facilities: (Secondary Barriers)	BL-1 plus: Autoclave available
BSL-2	BSL-2 practices plus: - Controlled access – Required disposable lab coats autoclaved after use - Equipment decontaminated before removed. Associated with human disease; special precautions required for some agents.

C. Biohazard Definition

Infectious or etiologic (disease causing) agents, potentially infectious materials, certain toxins, and other hazardous biological materials are included in the definition of a biohazard.

Biohazard

Biological agents and materials which are potentially hazardous to humans, animals, and/or plants. Biohazardous agents may include but are not limited to:

Certain bacteria, fungi, viruses, *rickettsiae*, *chlamydiae*, parasites, recombinant products, allergens, cultured human or animal cells, and the potentially infectious agents these cells may contain viroid's, prions, and other infectious agents as outlined in laws, regulations, or guidelines.

Biohazards at KGI

Biological hazards can be found in the various research and support environments on campus. Current projects at KGI are dealing with infectious agents BLS-L1 and L2 only. However, despite having biohazards on campus, KGI is a safe place to work, teach, and learn. The information contained in this and other manuals will further increase our ability to maintain a safe and healthy work environment.

D. Classification of Infectious Agents

Risk Groups

Worldwide there are several systems for classifying human and animal pathogens according to the hazard they present to an individual and the community. Although these classifications differ from each other, they all are based on the notion that some microorganisms are more hazardous than others. In general, the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures, and/or effective treatment is some of the criteria taken into consideration when classifying infectious agents. In the U.S., the most current classification is found in the ***NIH Guidelines for Research Involving Recombinant DNA Molecules***. The human etiologic agents addressed in these guidelines are classified into four risk groups with **Risk Group 1 (RG-1)** of low or no hazard and **Risk Group 4 (RG-4)** representing highly infectious agents:

osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

Table 2. 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 is not associated with disease in healthy adult humans.
Risk Group 2 (RG-2)	Agents that is associated with human disease which are rarely serious and for which preventive or therapeutic interventions are often available.
Risk Group 3 (RG-3)	Agents that is 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 realize, however, that none of the lists are inclusive. In addition, those agents not listed in Risk Groups 2, 3, and 4 are **not** automatically or implicitly 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 and their relationship to agents that are listed.

Risk Groups = Biosafety Level?

Determining the RG of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BL-2, and RG-3 agents at BL-3. However, the use of certain RG-2 agents in large quantities might require BL-3 conditions, while some RG-3 agents (such as the HIV virus) may be safely manipulated at a BL-2 under certain conditions. It is also true that some RG-2 agents can be handled at a BL-1 level. For more information, refer to the section on risk assessment or contact the Biological Safety Officer.

E. Rules, Regulations, and 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.

1. **National Institute 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 (rDNA) molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. As a result of the committee's activity, the initial version of the NIH Guidelines was published in 1976. It has been amended and revised many times since then. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Program with authority to approve or disapprove proposed rDNA research using the NIH Guidelines as a minimum standard. For more information, please refer to the Recombinant DNA Research section in this manual and the NIH Guidelines for Research Involving Recombinant DNA Molecules.
2. **Centers for Disease Control and Prevention (CDC)** and the **National Institute of Health (NIH) Guidelines on:** ***Biosafety in Microbiological and Biomedical Laboratories*** *(BMBL) 5 Th edition, Feb 2007*. In 1984, the CDC/NIH published the first edition of the BMBL. 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. KGI is using the BMBL and the Biosafety Manual from Oregon State University as the basis for this biosafety manual.
3. **Occupational Safety and Health Administration: Blood-borne Pathogens Standard.** In 1922, 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.
4. **Department of Health and Human Services (HHS - 42CFR 73)**: ***Possession, Use, and Transfer of Select Agents and Toxins.*** In 2002, HHS published a set of rules that require facilities and institutions to be registered and approved in order to possess, use, or transfer certain biological agents and toxins. HHS requires KGI to comply with the BMBL (see above) and OSHA's Laboratory Safety Standard 29 CFR 1910.1450.
5. Packaging, shipment, and transportation requirements for infectious substances, diagnostic specimens, and biological products are addressed in the following rules and guidelines:

Agencies:

- **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*
- **US Public Health Service**: *42 CFR Part 72*
- **US Postal Service**: *39 CFR Part 111*
- **US Department of Labor, OSHA**: *29 CFR 1910.1030*

6. Importation permits are required for certain infectious agents, biological materials, and animals as outlined in **US 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 by *7 CFR Part 340*.

F. Practices and Procedures

Routes of Infection

Working in a biological research environment like some labs at KGI, it is not unreasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

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 literature point to accidents during work with some type of infectious agent. These include spills, splashes, and accidents involving needles or other sharp objects. The general laboratory procedures outlined in this manual address those issues and provide for guidance in handling infectious or potentially infectious materials.

Administrative Controls

Biohazard Warning Sign

A biohazard label is required for all areas or equipment in which RG-2 or 3 agents are handled or stored or where BL-2 or 3 procedures are required. The appropriate place for posting the label is at the main entrance door(s) to laboratories and, on equipment like refrigerators, incubators, transport containers, and/or lab benches.

Training

Good microbiological and laboratory practices are essential for a safe work environment. All personnel working with RG-2 agents or at BL-2 should receive adequate laboratory specific training from the Principal Investigator (PI) or laboratory supervisor. Training should include at a minimum:

- Good laboratory and animal practices as applicable
- Site specific information on risks, hazards, and procedures
- Laboratory or environment specific bl-2 procedures as applicable

In addition, it is **mandatory** that all personnel working with select agents or at BL-2 handling RG-2 agents should attend biosafety training as identified by the BSO.

Blood-borne Pathogens Program

Please see the Exposure Control Plan (ECP).

Safe Housekeeping Guidelines

The **Occupational Safety and Health Act (OSH Act)** requires employers to comply with hazard-specific safety and health standards. In addition, pursuant to *Section5(a)(1)* of the OSH Act, often referred to as the General Duty Clause, employers must provide their employees with a workplace free from recognized hazards likely to cause death or serious physical harm. OSHA has previously used the General Duty Clause to cite employers for exposing employees to potential physical harm OSHA's General Requirements for Housekeeping related to Walking-Working Surfaces, *CFR 1910.22*, applies to all permanent places of employment. Good housekeeping reduces injuries and accidents, improves morale, reduces fire potential, and can even make operations more efficient. The following example is taken from OSHA's *Standard on Employee Emergency Plans and Fire Prevention Plans: 29 CFR 1910.38 (b) (3) Housekeeping*:

"The employer shall control accumulations of flammable and combustible waste materials and residues so that they do not contribute to a fire emergency." Proper housekeeping as a method of fire control is also covered in *1910.37 (a) (3)*, requiring that exit routes must be free and unobstructed. No materials or equipment may be placed, either permanently or temporarily, within the exit route.

Housekeeping

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

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

- Keeping the laboratory neat and free of clutter—surfaces should be clean and free of infrequently used chemicals, glassware, and equipment.
- Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes—old and unused chemicals should be disposed of promptly and properly.
- Providing a workplace that is free of physical hazards—aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, and avoidance of the creation of electrical hazards in wet areas.
- Remove unnecessary items on floors, under benches. or in corners.
- Properly secure all compressed gas cylinders.
- Never use fume hoods for storage of chemicals or other materials.
- Do not use Bunsen burners in Biological Safety Cabinets.
- Promptly remove all autoclaved items from the autoclave room when sterilization is complete.
- All autoclave bags must have red biohazard bag for sterilization, labeled with Name and Date written across autoclave tape. After sterilization, add a waste label to the red bag and place the red bag into a black trash bag for outside disposal.
- Remaining in compliance with KGI's Chemical Hygiene Plan.
- All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance.

Engineering Controls

Biological Safety Cabinets (BSCs)

BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed. Two kinds of biological safety cabinets, designated as Class I and II, have been developed to meet various research and clinical needs. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their

exhaust and/or supply systems. Biological safety cabinets must not be confused with other laminar flow devices or “clean benches”; in particular, horizontal flow cabinets which direct air towards the operator and should never be used for handling infectious, toxic, or sensitizing materials.

Laboratory personnel must be trained in the correct use and maintenance of biological safety cabinets to ensure that personnel and product protection (where applicable) are maintained.

1. Class I Biological Safety Cabinet

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

2. Class II Biological Safety Cabinet

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

3. Class III Biological Safety Cabinet (not currently available at KGI)

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

Biological safety cabinets, when properly used in research and teaching activities involving the manipulation of biohazardous agents, are effective in containing and controlling particulates and aerosols and complement good laboratory practices and procedures. The correct location, installation, and certification of the biological safety cabinets are critical to its performance in containing infectious aerosols. All BSCs used for RG-1 or 2 and rDNA research are inspected annually and certified by trained and certified service personnel according to the NSF (National Sanitation Foundation) Standard 49. Inspection and re-certification are mandatory if the cabinet is relocated or after major repairs, filter changes etc. To request service or certification contact, Jasmine Yu at extension 78698.

Safe and Effective Use of Biosafety Cabinets

1. General:

- Make sure the BSC is certified (NSF sticker) when it is installed or after it is moved, or repaired and annually thereafter. Check the gauge or electronic controls regularly for any indication of a problem.
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking rapidly behind you, and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.
- Plan your work.
- Minimize the storage of materials in and around the BSC.
- Allow cabinet to run for 10-15 minutes before shutting off.

2. Operation:

- Before using, wipe work surface with 70% alcohol or any other disinfectant suitable for the agent(s) in use. Wipe off each item you need for your procedures before placing it inside cabinet.
- **Do not** place any objects over the front air intake grille. **Do not** block the rear exhaust grille.
- Segregate contaminated and clean items. Work from "clean to dirty."
- Place a pan with disinfectant and/or sharps container inside the BSC for pipette discard. **DO NOT** use vertical pipette discard canisters on the floor outside the cabinet.
- Move arms slowly when removing or introducing new items into the BSC.
- If you use a piece of equipment that creates air turbulence in the BSC (such as a micro-centrifuge, blender), place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
- Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.
- Clean up spills in the cabinet immediately. Wait 10 minutes before resuming work.
- When work is finished, remove all materials and wipe all interior surfaces with 70% alcohol or any other disinfectant suitable for the agent(s) in use.
- Remove lab coat, gloves, and other Personal Protective Equipment (PPE) and wash hands thoroughly before leaving the laboratory.

Safety Equipment

1. **Safety showers** provide an immediate water drench of an affected person. Standards for location, design, and maintenance of safety showers are outlined in the CHP.
2. **Eyewash stations** are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially infectious materials. Standards for location, design, and maintenance of emergency eyewash facilities are outlined in the CHP.
3. **Ventilation controls** are those controls intended to minimize employee exposure to hazardous chemicals and infectious or toxic substances by removing air contaminants from the work site. There are two main types of ventilation controls:
    1. **General (Dilution) Exhaust**: a room or building-wide system which brings in air from outside and ventilates within. Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work. General exhaust systems are inadequate for RG-3 agents or BL-3 work.
    2. **Local Exhaust or Filtration**: a ventilated, enclosed work space intended to capture, contain, and exhaust or filter harmful or dangerous fumes, vapors, and particulate matter. In the case of hazardous chemicals this includes a fume hood. In the case of infectious agents BSCs should be used. For more information on ventilation requirements involving hazardous chemicals refer to the CHP.

Personal Protective Equipment (PPE)

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

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

Laboratory Clothing: At a minimum, a laboratory coat should be worn in all laboratories. A disposable lab coat will be provided for all personnel working in BL-2 labs. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties, and durability. Protective clothing must be removed and left in the laboratory before leaving for non- laboratory areas. Disposable clothing should be available for visitors, maintenance, and service workers in the event it is required. Dirty protective clothing should be discarded in the appropriate “dirty laundry hampers.” Hampers are located near clean lab coats in Building 517 and 535. Personnel must not take laboratory clothing home.

Gloves: Must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances, hazardous

chemicals, and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required.

Respirators: KGI currently has a respirator program in place for the use of NP95 mask. Training and fitting will be done by a trained KGI Safety Officer. A medical exam is also required prior to using a respirator. Please see the BSO for the medical referral form.

Shoes: Always wear comfortable and safe low profile shoes. Open toed shoes, sandals, and flip-flops are prohibited when working in any lab area.

Skirts and Shorts: A lab coat should be worn when wearing a skirt or shorts which may expose your bare legs to chemicals or biological agents.

Recommended Work Practices

Pipettes and Pipette Aids

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

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette.
- Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to comp letely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a biosafety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet as well while in use.

Syringes and Needles

Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. Do not use a syringe and needle as a substitute for a pipette.

Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe.

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

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

Needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe following use. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one-handed scoop method must be used. Always dispose of needle and syringe unit promptly into an approved sharps container.

Centrifuge Equipment

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

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

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings, and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors, and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
- Always balance buckets, tubes, and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when suspending sediment material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.

- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion, and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

Ultrasonic Disrupters, and Lyophilizers

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

Lyophilizers and Ampoules

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

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

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

Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended. Never use a Bunsen burner in a biological or chemical safety cabinet.

Biohazard Spill Cleanup Procedures

All biological spills at or above BSL-2 level should be reported to **Jasmine Yu at extension 78698**, no matter what size. Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards must have a basic biological spill kit ready to use at all times. Materials should be easily accessible to everyone in the lab. If your BSL- 2 lab does not have a spill kit, please contact the BSO officer.

The following procedures are provided as a guideline to biohazardous spill cleanup and will need to be modified for specific situations. **As with any emergency situation, stay calm, call 911 if necessary, and proceed with common sense**. Call KGI Spill Team if further assistance is required, especially if the spill outgrows the resources in the laboratory.

Spill Inside the Laboratory (BL-1, RG-1)

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further processing. Wear a disposable gown or lab coat, safety goggles, and gloves. Initiate cleanup with disinfectant as follows:

Have a complete biological spill kit ready to go BEFORE you start the cleanup

- Cover spill with paper towels or other absorbent material containing disinfectant.
- Encircle the spill with disinfectant (if feasible and necessary), being careful to minimize aerosols.
- Decontaminate and remove all items within spill area.
- Remove broken glassware with forceps or broom and dustpan and dispose in sharps container. Do not pick up any contaminated sharp object with your hands.
- Remove paper towels and any other absorbent material and dispose in biohazard bags.
- Apply disinfectant to the spill area and allow for at least 10 minutes contact time to ensure germicidal action of disinfectant.
- Remove disinfectant with paper towels or other absorbent material and dispose of in biohazard bag.
- Wipe off any residual spilled material and reapply disinfectant before final cleanup.
- Wipe equipment with equipment compatible disinfectant (e.g., non-corrosive). Rinse with water if necessary.

- Place disposable contaminated spill materials in biohazard bags for autoclaving.
- Place contaminated reusable items in biohazard bags or heat resistant pans or containers with lids before autoclaving.
- Reopen area to general use only after spill cleanup and decontamination is complete.
- Inform all personnel and laboratory supervisor about the spill and successful cleanup as soon as possible.

Spill Inside the Biological Safety Cabinet (BL-2, RG-2)

Have a complete biological spill kit ready to go BEFORE you start the cleanup

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

General Guidelines and Policies

Biological Risk Assessment

The assessment of risk is an essential element of safety in the laboratory. For most situations, guidelines, rules, and regulations have clearly defined the procedures and practices to be followed in order to achieve safety in the work place. However, the newly isolated agent or toxin, or the new procedure never before employed needs further evaluation. Questions concerning the appropriate safety equipment, training, and waste disposal need to be addressed as well as safe procedures and practices. Something is considered safe if the risk associated with it is judged to be acceptable. However, since individual judgment involves both personal and social values, opinions on what is safe or not can vary significantly. In order to find a common ground for an acceptable risk assessment, the "rule of reason" needs to be applied. The following factors should be considered for the determination of reasonableness:

1. **Custom of usage (or prevailing professional practice)**: Many laboratory procedures involve the maintenance of sterility and cleanliness. These procedures are commonly considered safe, since adverse effects would have been obvious over time. However, because a procedure has been used for many years do not necessarily imply that it is safe. The best example is mouth-pipetting, which was used for centuries and finally considered a very dangerous procedure and habit.
2. **Best available practice, highest practicable protection, and lowest practicable exposure**: It should be common practice in the microbiological laboratory to use the best available procedures with the highest level of protection. This not only provides for a safe work environment but also fosters excellence in scientific conduct.
3. **Degree of necessity or benefit**: The common question to ask is, are the benefits worth the risk? There is no need to use a human pathogen causing severe gastroenteritis in a teaching laboratory when principal microbiological practices can be taught with an organism that is not considered to be infectious.
4. **No detectable adverse effects**: This can be a very weak criterion since it involves uncertainty or even ignorance.
5. **Principal knowledge**: A lot of times, existing procedures are modified, involving the same or similar toxic chemicals or agents. For that reason, similar safety procedures should be applied. If new agents are isolated, we need to ask what we know about the close relatives. Many agents of known etiologic character are already categorized in risk groups allowing for the selection of the appropriate biosafety level. New isolates from infected animals or humans with known infectious relatives warrant at a minimum the same level of protection.

Taking the above mentioned factors, as well as others, into consideration will allow for a reasonable approach to a new challenge. KGI's Biological and Chemical Safety Officer is available to assist in this process and should be contacted for questions concerning chemical and biological safety. Once a risk assessment is completed, the results should be

communicated to everyone involved in the process. Written standard operating procedures (SOPs) should be established and distributed.

Guidelines for Working with Tissue Culture/Cell Lines

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same BS level as that recommended for the agent.

The Centers for Disease Control and Prevention (CDC) and OSHA recommend that all cell lines of human origin be handled at BSL-2. All personnel working with or handling these materials need to be included in KGI's Exposure Control Plan, developed by the laboratory PI.

Cell lines which are non-primate or are of normal primate origin, which do not harbor a primate virus, which are not contaminated with bacteria, mycoplasma or fungi and which are well established may be considered Class I cell lines and handled at a Biosafety Level 1. Appropriate tests should confirm this assessment.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until shown to be free of all adventitious agents), and all virus and mycoplasma-containing primate cell lines are classified as RG-2 and should be handled at a Biosafety Level. Studies involving suspensions of HIV prepared from T-cell lines must be handled at BL-3.

Guidelines for Preventing the Transmission of Tuberculosis

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30 year downward trend. Recently, drug resistant strains of *Mycobacterium tuberculosis* have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of healthcare workers have died.

In December 2005, CDC published its *Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities*. The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance, and training for those personnel who are considered at risk for exposure to tuberculosis.

Guidelines for Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at BL-2.

A primary barrier, such as a biological safety cabinet, should be used when it is anticipated that splashing, spraying, or splattering of clinical materials may occur, for initial processing

of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis*), to protect the integrity of the specimen.

The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented. Additional recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards (NCCLS).

Use of Human Subjects and Materials

Federal and KGI regulations and policies require that all research involving human subjects or materials be reviewed and approved before initiation by the Institutional Review Board (IRB) to protect the rights and welfare of human subjects. Please see the BSO officer for details.

Transportation of Biological Materials

All biological materials should be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release and endangerment of the public and the environment. KGI has strict guidelines for transporting either into or away from its facility.

- **Transportation in-between buildings or locations on and off campus roads:**
  - KGI does not permit such materials to be transported off campus without following strict guidelines set forth by OSHA and DOT.
  - All samples transported intra-and inter buildings will be secured in durable leak proof containers of polycarbonate which meets OSHA *Standard 29 CFR Part 1910.1030*. This container will then be placed in a plastic outer bin with the appropriate Biohazard stickers on all four sides.
- **Transportation and shipment via carrier off campus:**
  - The shipment of diagnostic and clinical specimens, biological products, infectious agents, and recombinant DNA molecules is regulated by national and international transportation rules *CFR 49*. This includes specific procedures for the correct packing and packaging of these materials, necessary documentation, and labeling and permits. Please contact **Jasmine Yu at extension 78698** before shipping and or receiving these materials.

Decontamination

Methods of Decontamination: Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

In order to select the proper method and tools, it is important to consider, for example, the following aspects:

- Type of biohazardous agents, concentration, and potential for exposure.
- Physical and chemical hazards to products, materials, environment, and personnel.

Physical and chemical means of decontamination fall into four main categories:

- Heat
- Liquid chemicals

Choosing the right concentration and incubation time

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

Heat

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

Liquid Chemicals Used as Disinfectants

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency,

depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:

- **Nature of surface being disinfected**: Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to be effective.
- **Number of microorganism present**: Higher concentrations requires a longer application time and/or higher concentration of disinfectant.
- **Resistance of microorganisms**: Microbial agents can be classified according to increasing resistance to disinfectants and heat (see Table 3).
- **Presence of organic material**: The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- **Duration of exposure and temperature**: Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

Table 3. Resistance to Chemical Disinfectants

		Examples
Least Resistant ↓ **Most Resistant**	LIPID OR MEDIUM-SIZE VIRUSES	Herpes simplex virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus Human Immunodeficiency virus
	VEGETATIVE BACTERIA	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella choleraesuis*
	FUNGI	*Trichophyton sp.* *Cryptococcus sp.* *Candida sp.*
	NONLIPID OR SMALL VIRUSES	*Poliovirus* *Coxsackievirus* *Rhinovirus*
	MYCOBACTERIA	*Mycobacterium tuberculosis* *M. bovis*
	BACTERIAL SPORES	*Bacillus subtilis,* *Clostridium sporogenes*

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines.

Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are discussed below:

Alcohols: Ethyl or isopropyl alcohol in concentration of 10% to 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. Concentrations above 70% are less effective.

Formalin: Formalin is 37% solution of formaldehyde in water. Dilution of formalin to 5% results in an effective disinfectant. Formaldehyde is a 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 glutaraldehyde are irritating to the eyes, nasal passages, and upper respiratory tract. They should be used always 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 mostly from 5-10%. 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 to disinfect 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. Diluted solutions may be kept for extended periods if kept in a closed container and protected from light. However, it is recommended to use freshly prepared solutions for spill clean-up purposes. Chlorine-containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. 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.

Vapors and Gases: A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility.

Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like biological safety cabinets. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde.

Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

Biohazard Waste

At KGI, the term **biohazardous waste** is used to describe different types of waste that might include infectious agents. Currently, the following waste categories are all considered to be biohazardous waste:

Medical waste: Solid waste which is generated in the diagnosis, treatment (e.g., provision of medical services), or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, and includes:

1. Cultures and stocks of infectious agents and associated biologicals, including laboratory waste, biological production waste, discarded live and attenuated vaccines, culture dishes, and related devices.
2. Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or materials stained with blood or body fluids.
3. Pathological waste: defined as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
4. Sharps: Defined as needles, syringes, scalpels, lancets, and intravenous tubing with needles attached regardless of whether they are contaminated or not.
5. Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.

Regulated waste

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

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

General Labeling, Packaging, and Disposal Procedures

Currently, biohazardous waste is to be decontaminated before leaving KGI. Most of the waste can be autoclaved prior to disposal, while some waste may be incinerated. The responsibility for decontamination and proper disposal of biohazardous waste lies with the producing facility (e.g., laboratory and department).

- All biohazardous waste needs to be packaged, contained, and located in a way that protects and prevents the waste from release at any time at the producing facility prior to ultimate disposal. If storage is necessary, putrefaction and the release of infectious agents in the air must be prevented.
- If not stated otherwise (see below), most biohazardous waste will be disposed of in red autoclaved biohazard bags. KGI requires the use of red biohazard bags that includes the biohazard symbol. Please use auto clave waste disposal tags provided by the Biological Safety Officer. The tags should be filled out completely. All waste disposed of in these bags is to be autoclaved until the waste is decontaminated. Please refer to the Autoclave Training Document on the KGI safety website.

- KGI has a permit issued by the California State Health Department qualifying it as an Official On-site Medical waste generator as such; after successful autoclaving (decontamination), all red biohazard bags need to be bagged in (black) plastic non-biohazard bags that are leakproof. These (black) bags can be put in dumpsters in the back of Building 535 and for Building 517 the dumpster east of the building for pickup by mainstream waste disposal services. Biohazardous waste that has been successfully decontaminated by autoclaving is no longer considered hazardous.
- Since autoclaves are an integral part of KGI's biohazardous waste treatment procedure, proper operation and maintenance is very important. All users of autoclaves need to be trained in the proper operating procedures by Laboratory Safety Manager, Jasmine Yu. Maintenance and repair of autoclaves used for the decontamination of biohazardous waste are the responsibility of KGI's facilities and Institutional Laboratory Support.

Waste Specific Procedures for BL-1 and BL-2

Cultures, Stocks, and Related Materials

Cultures and stocks of BSL2 infectious agents and associated biologicals (as defined above), can be sterilized by adding a 1:10 bleach to water and letting sit 15 minutes after which it can then be disposed of in the regular sewer system using copious amounts of water.

Bulk Liquid Waste, Blood, and Blood Products

Sharps

- **All biohazard sharps** must be placed in a red rigid, puncture resistant, and closable and leak proof container, which is labeled with the word "Sharps" and the biohazard symbol.
- **All non-biohazard sharps** must be placed in a clear or yellow rigid, puncture resistant, closable and leak proof container, which is labeled with the word "Sharps" and the word non-hazardous material.
- **Food containers** (e.g., empty coffee cans) are not permissible as sharps containers. All sharps must be handled with extreme caution. The clipping, breaking, and recapping of needles is not recommended. Sharps containers should not be filled more than 2/3. After use, the container needs to be closed and labeled with a KGI Hazardous Materials Label.

Contaminated Solid Waste

- Contaminated solid waste includes cloth, plastic, agar plates, and paper items that have been exposed to agents infectious or hazardous to humans, animals, or plants. These contaminated items shall be placed in red biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags. **Contaminated Pasteur pipettes are considered sharps** and need to be disposed of

in a sharps container. After autoclaving, the red bags must be placed in black trash bags before disposing in the dumpster.

Waste Specific Procedures for Biosafety Level 2 (BL-2)

All bio-hazardous waste including RG 2 agents that are handled at BL-2 is to be autoclaved at the point of origin (laboratory, or facility). Transportation of non-autoclaved BL-2 waste outside of the building is by placing the waste into a rigid leak-proof container with a tight-fitting lid. The container should have the standard Biological Safety Sticker on three sides and the top. If your lab does not have such a container, please see the Biological Safety Officer.

Decontamination of Biohazardous Waste by Autoclaving

- Autoclaving is accepted as a safe and effective procedure for sterilization.

  A steam sterilization integrator strip is used to indicate pressure, moisture, and time. PLEASE make sure that all waste has this tape attached with the lab number indicated on the tape.

Autoclave Procedures:

- Strong oxidizing material (chemicals) must not be autoclaved with organic material: Oxidizer + Organic Material + Heat = Possible Explosion.
- All biohazardous waste must be placed in red biohazard bags with a heat sensitive "Autoclaved" tape indicator.
- Prior to autoclaving, a red biohazard bag containing waste must be kept closed to prevent airborne contamination and nuisance odors. Preferably in a closed waste container.
- When autoclaving, the red bags must be tied in such a way as to allow steam to penetrate the waste therein. Upon removal of the bag from the autoclave, it should be closed and put into a black trash bag to be disposed of in trash bin behind Building 535, and the east corner parking lot of Building 517.
- It is recommended to add 100ml to 200ml water to each biohazard before autoclaving.
- Autoclave biohazardous materials for at least 40 minutes at the standard 121°C/250°F and 15 PSI for a single bag and at least 60 minutes for a run with numerous bags.

G. Investigation, Analysis, Incident/Near Miss Report, and Corrective Action

Scope of Investigation and Analysis

A near-miss incident is an unplanned event that did not result in injury, illness, or damage—but had the potential to do so. Only a fortunate break in the chain of events prevented an injury, fatality, or damage. Although human error is commonly an initiating event, a faulty process or system invariably permits or compounds the harm, and should be the focus of improvement.

Other familiar terms for these events is a “close call”, or in the case of moving objects, “near collision”. The often misunderstood phrase is so-called to stress that not only had things gone remotely off course towards danger, but they had actually only “barely missed” catastrophe. Near-misses are smaller in scale, relatively simpler to analyze, and easier to resolve. Thus, capturing near-misses not only provides an inexpensive means of learning it has some equally beneficial spin offs.

1. Captures sufficient data for statistical analysis; trending studies.
2. Provides immense opportunity for ‘employee participation’ a basic requirement for successful Chemical and Biological Laboratory Safety Program. This embodies principles of behavior shift, responsibility sharing, awareness, and incentives etc.

- Near Miss A Zero Cost Learning Tool: A near-miss is a cheaper learning tool than learning from actual injury or property loss accident.
- An ideal near miss event reporting system includes both mandatory (for incidents with high loss potential) and voluntary, (non-punitive reporting by witnesses). A key to any near-miss report is the “lesson learned”. Near-miss reporters are in a position to describe what they observed about genesis of the event, and the factors that prevented loss from occurring.
- The events that caused the near-miss are subjected to root cause analysis to identify the defect in the system that resulted in the error and factors that may either amplify or ameliorate the result.
- To prevent the near-miss from happening again, the organization must institute teamwork training, feedback on performance, and a commitment to continued data collection and analysis; a process called continuous improvement.

General process of investigation and analysis for documenting a Corrective Action

Incident/ Near Miss forms are the most critical part of successful corrective action, because it directs the corrective action at the root of the problem. That is to say, it is effective solutions we seek, not root causes. Root causes are secondary to the goal of prevention, and are only revealed after we decide which solutions to implement.

1. Define the problem.
2. Gather data/evidence through reports, interviews, and investigation of laboratory or facilities involved in the incident.
3. Ask why and identify the causal relationships associated with the defined problem.
4. Identify which causes if removed or changed will prevent recurrence.
5. Identify effective solutions that prevent recurrence, that are within your control, meet your goals and objectives, and do not cause other problems.
6. Implement the recommendations.
7. Observe the recommended solutions to ensure effectiveness.
8. Apply Root Cause Analysis (RCA) to aid in investigation and final analysis.

Analysis Tool for Investigation (root cause analysis RCA)

Root cause analysis (RCA) is a class of problem-solving methods aimed at identifying the root causes of problems or events. The practice of RCA is predicated on the belief that problems are best solved by attempting to correct or eliminate root causes, as opposed to merely addressing the immediately obvious symptoms. By directing corrective measures at root causes, it is hoped that the likelihood of problem recurrence will be minimized. However, it is recognized that complete prevention of recurrence by a single intervention is not always possible. Thus, RCA is often considered to be an iterative process, and is frequently viewed as a tool of continuous improvement.

RCA, initially, is a reactive method of problem detection and solving. This means that the analysis is done after an event has occurred. By gaining expertise in RCA it becomes a pro-active method. This means that RCA is able to forecast the possibility of an event even before it could occur. Root cause analysis is not a single, sharply defined methodology; there are many different tools, processes, and philosophies of RCA in existence. However, most of these can be classed into f ve, very-broadly defined “schools” that are named here by their basic fields of origin: safety-based, production-based, process-based, failure-based, and systems-based.

- Safety-based RCA descends from the fields of accident analysis and occupational safety and health.
- Production-based RCA has its origins in the field of quality control for industrial manufacturing.
- Process-based RCA is basically follow-on to production-based RCA, but with a scope that has been expanded to include business processes.
- Failure-based RCA is rooted in the practice of failure analysis as employed in engineering and maintenance.

- Systems-based RCA has emerged as an amalgamation of the preceding schools, along with ideas taken from fields such as change management, risk management, and systems analysis.

Incident Reporting and Procedures

1. All incidents/Near misses no matter how minor, may need to be reported to the BSO (please use common sense and reasonableness in evaluating a near-miss); often times a near-miss even a minor one adds valuable insight to prevent future larger incidents. For clarification of a nea- miss please refer back to the previous section on (Scope of Investigation and Analysis)
2. The BSO must start an investigation beginning with an initial lab inspection.
3. Each person involved in the incident must fill out an Incident Report provided by the BSO. The reports must be completed within five (5) business days following the reporting.
4. If deemed necessary, the BSO may require an interview from each lab member.
5. Once the interviews and incident reports are complete, the BSO may require additional information from any lab member if needed in order to collect sufficient data to provide efficient corrective actions.
6. The BSO will deliver a full report to the President, Dean, and Human Resource Manager.
7. The BSO will provide the PI and lab members with a list of corrective actions, timelines for completion, and follow up dates for a final inspection.
8. The President and Dean will enforce compliance of all corrective actions set forth by the BSO if required.

H. Recombinant DNA Research

All recombinant DNA/RNA, select Agent, and GMO Research must be approved by the Intuitional Biosafety Committee (IBC).

The IBC provides guidelines and procedures for the Research. Please see:

"Notice pertinent to the March 2013 revisions, of the NIH guidelines for research involving recombinant or synthetic nucleic acid molecules (NIH guidelines)"
osp.od.nih.gov/biotechnology/nih-guidelines

KGI relies on Western University as its IBC of record. The Western University IBC protocol form, instructions, and additional information can be found here:
westernu.edu/research/regulatory-affairs/research-biosafety

David Sanchez, PhD, is the current Chair of Western Univeristy's IBC. Dr. Sanchez can be reached at 909.469.8479 or via email at sanchezd@westernu.edu. The KGI Lab Safety Committee serves as the liaison between KGI and the IBC at Western University. Please contact either Larry Grill at 909.607.0175 or Jasmine Yu at 909.607.8698 if you have questions on IBC protocols.

I. References

1. CDC Centers for Disease Control
   cdc.gov/niosh
2. The Occupational Safety and Health Administration (OSHA) promulgated a regulation entitled Occupational Exposure to Hazardous Chemicals in Laboratories, otherwise known as the Laboratory Standard.
3. The goal of the Lab Standard is to ensure that laboratory workers are informed about the hazards of chemicals in their workplace and are protected from chemical exposures exceeding allowable levels (e.g., OSHA Permissible Exposure Limits).
4. All individuals who work with hazardous chemicals in science and engineering laboratories are obligated to comply with the Lab Standard. Work with chemicals outside of laboratories is covered by the OSHA Hazard Communication Standard.
5. PPE, Personal Protective Equipment
   osha.gov/laws-regs/regulations/standardnumber/1910/1910.133
6. NIOSH Databases and Other Resources
   cdc.gov/niosh/topics/chemical-safety/#data
7. Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities
   cdc.gov/mmwr/pdf/rr/rr5417.pdf
8. MSDS-Search
   msds.com

Special thanks to Oregon State University for the framework to which KGI built its BSO manual.