California Institute of Technology

BIOSAFETY MANUAL

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BIOSAFETY MANUAL

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CHAPTER I: INTRODUCTION

The purpose of the California Institute of Technology's (Caltech) Biosafety Manual is to increase awareness of biological hazards frequently encountered in research and teaching laboratories at Caltech and to provide guidance on recommended practices.

The Biosafety Manual is designed in accordance with *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and the Biosafety in Microbiological and Biomedical Laboratories (BMBL, 5th Edition) to assure laboratory personnel that – with proper precautions, equipment, and facilities – most biohazardous materials can be handled without undue risk to themselves, their associates, their families, or the environment.

This Manual is intended for trained microbiologists as well as individuals handling biohazardous material in other laboratory disciplines such as bioengineering, chemical engineering, applied science, oncology, immunology, or molecular biology. Persons who have little research laboratory training might not realize the potential hazard involved with their materials, and should seek additional information.

The safety principles described herein are based on sound safety practices, common sense, current data, good housekeeping, thorough personal hygiene, and tested accident-response plans.

Laboratories that are well organized and procedurally disciplined are proven not only safe, but also scientifically effective.

CHAPTER II: Caltech's Code of Conduct

Caltech's Code of Conduct embodies overarching principles for which each scientist is accountable for in their work and their labs. As members of the Caltech community, we expect that each of us will put in practice the high standards that have gained Caltech its worldwide reputation (http://codeofconduct.caltech.edu/).

The Caltech code of conduct is centered on:

- Commitment to excellence, accountability, honesty and respectful discourse, curiosity and exploration, dignity and respect, education, stewardship to the Institute resources, integrity, fostering of a safe environment, transparency, and leadership.

In the realm of research involving biological hazardous material, pathogens and toxins, additional responsibilities include:

- Awareness of and adherence to all safety and security protocols;
- Knowledge and awareness of spill and exposure response protocols;
- Knowledge of and adherence to reporting requirements related to spills or potential exposures;
- Knowledge and awareness of all emergency response protocols (e.g., fire, earthquake);
- Completion of all training requirements (lectures and hands-on);
- Completion of all Occupational Health requirements, including documentation of required physicals, medical clearances, and/or vaccinations, when applicable;
- **Immediate reporting to the Principal Investigator of any situation that compromises an individual's ability to perform as required in a BSL-2 or ABSL-2 laboratory, including physical or psychological issues; and**
- **Immediate reporting to the Principal Investigator and the Institute, where appropriate, of behavior or activities that are inconsistent with Institute safety and security plans.**

Institutional support for the scientist in discharging the above responsibilities is essential. At the individual level, one form of such support is the Caltech Staff and Faculty Consultation Center (SFCC - http://sfcc.caltech.edu/). The SFCC is a confidential service that provides support, counseling, referrals, and resources for issues that impact your life and potentially compromise your ability to perform safely in the laboratory. Registered students may also seek help through the Caltech Counseling Service (https://counseling.caltech.edu/).

Another important institutional mechanism is a formal, anonymous reporting mechanism for instances of noncompliance in the laboratory with established Caltech safety and/or security policies. At Caltech, multiple

pathways exist whereby behaviors of concern can be anonymously reported, depending on the particular situation at hand. Included among these options are: (1) Reporting to your PI/Supervisor; (2) Reporting to your Department Administrator and/or Chair; (3) Reporting to the Caltech Campus Anonymous Hotline (http://hotline.caltech.edu/ x8787); (4) Reporting to the Institute Biosafety Officer, the Research Compliance Director, or the Institutional Biosafety Committee; (5) Reporting to the Environmental, Health, and Safety Office.

ROLES & RESPONSIBILITIES

Principal Investigator
The Principal Investigator (PI) is responsible for the health and safety of laboratory personnel doing work in their laboratory. The PI may delegate the safety duties, but not the responsibility, and must make sure that all safety duties are carried out in a consistent manner.

The PI must:

- Comply with all applicable state and federal regulations and guidelines
- Ensure the safe operation of their laboratory
- Assess the risks of their ongoing or planned experiments and convey those risks to lab personnel
- Provide lab-specific orientation to new lab personnel including the location of the Emergency Response Guide and emergency equipment located in the laboratory
- Register and obtain approval for experiments falling within the *NIH Guidelines* with the IBC, as well as other experiments falling under the purview of the IBC (https://ibc.caltech.edu/)

Laboratory Safety Officer
Each PI overseeing a research laboratory may designate one or more Laboratory Safety Officer(s). Duties may include but shall not be limited to:

- Promulgate biological safety information to other laboratory members.
- Provide lab-specific orientation to new lab members including the location of Emergency Response Guide and emergency equipment located in the laboratory.
- Act as a liaison with the Institute Biosafety Officer, the IBC, or the office of Research Compliance on behalf of the PI.
- Act as the lab emergency coordinator and liaison with the Caltech Emergency Response Network.
- Facilitate periodic, ongoing biosafety laboratory inspections.

Laboratory Personnel
All laboratory personnel, including students, who work with biohazardous materials in research laboratories are responsible for the following:

- Comply with safety rules, regulations, and procedures required for the task assigned.
- Know and understand the hazards of materials and processes prior to conducting work and utilize appropriate measures to control these hazards.
- Attend required training.
- Use appropriate personal protective equipment (PPE).
- Participate in medical surveillance when required.
- Report accidents, injuries, or near misses to the PI and Safety Office.
- Report unsafe conditions to the PI or immediate Supervisor, and Safety Office.
- Keep the work areas safe and uncluttered, and maintain appropriate hygiene practices.

Division Administrators

It is the responsibility of the Division Administrator to ensure their Division conducts operations in accordance with applicable laws and regulations, and to implement the Institute's environmental, safety, and emergency policies.

Additionally, Division Administrators are responsible for implementing Caltech's Injury and Illness Prevention Plan (IIPP) which includes:

- Ensuring that workplaces and equipment are safe, well maintained, and in compliance with external governmental regulatory agency regulations and Caltech's policies, procedures, programs, and practices;
- Ensuring that workplace safety and health practices and procedures are clearly communicated and understood by employees through training programs;
- Enforcing health and safety rules fairly and uniformly as they relate to job performance;
- Acknowledging employees who make a significant contribution to maintenance of a safe workplace, and disciplining employees who fail to follow safe work practices;
- Encouraging employees to report workplace hazards without fear of reprisal;
- Ensuring that periodic, scheduled workplace inspections/surveys are conducted, and that identified health and safety deficiencies are corrected in a reasonable time period;
- Ensuring that workplace incidents (i.e., injuries, exposures, or illnesses) are reported and investigated, and that corrective action is taken; and
- Ensuring that inspections/investigations and employee records are kept for the designated time period(s).

Institute Biosafety Officer

The Institute Biosafety Officer (BSO) is responsible for the following:

- Provide technical guidance on matters pertaining to biosafety;
- Prepare, administer, and oversee Institutional implementation of the Biosafety Manual;
- Periodically review the Biosafety Manual and revise it as necessary;
- Investigate accidents, illnesses, or near misses involving biohazardous materials;
- Provide and coordinate biosafety training including principles of Biosafety training, NIH Guidelines training, Bloodborne Pathogens training, Viral Vectors training, and Biological Toxins training;
- Perform observational surveys/laboratory inspections of laboratories and propose corrective actions.
- Assist PIs with risk assessments;
- Assist research staff with submission of registrations to the IBC and maintain registration files;
- Assist the Research Compliance Office with reporting recombinant or synthetic nucleic acid incidents and violations of the Guidelines to the IBC or, on behalf of the IBC to the NIH Office of Biotechnology Activities.
- Identify concerns or gaps in compliance; and
- Develop emergency and reporting procedures.

CHAPTER III: GENERAL BIOSAFETY PRINCIPLES

A. RISK ASSESSMENT

To apply the appropriate biological safety principles while handling a potential pathogen, one must first perform a risk assessment, which considers:

1. The biological and physical hazard characteristics of the agent,
2. The sources likely to harbor the agent,
3. The susceptibility of the potential incidental host,
4. The procedures that may disseminate the agent, and
5. The best method to effectively inactivate the agent.

Globally, numerous government agencies have classified microorganisms pathogenic for humans into risk groups (RG) based on the transmissibility, invasiveness, virulence or disease-causing capability, lethality of the specific pathogen, and the availability of vaccines or therapeutic interventions. Risk groupings of infectious agents usually correspond to biosafety levels (BSL), which describe recommended containment practices, safety equipment, and facility design features necessary to safely handle these pathogenic microorganisms. The list of pathogenic microorganisms includes bacteria, viruses, fungi, parasites, and other infectious entities. The scheme ascends in order of increasing hazard from Risk Group 1 (RG1) agents, which are nonpathogenic for healthy human adults, to RG4 agents, which display a high morbidity and mortality and for which treatments are not generally available.

RG1	Agents are not associated with disease in healthy adult humans; *Examples: Bacillus subtilis, E. coli K-12,* AAV.
RG2	Agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. *Examples: Staphylococcus aureus, Human Adenovirus, Salmonella spp.*
RG3	Agents are associated with serious or lethal human disease; for which preventive or therapeutic interventions may be available; *Examples: Mycobacterium tuberculosis, HIV, Yersinia pestis, Bacillus anthracis, Avian Influenza H1N*
RG4	Agents associated with serious or lethal human disease; for which preventive or therapeutic interventions are usually not available; *Examples: Ebola virus, Marburg virus, Lassa virus, and Herpes B virus*

The risk group listing of the *NIH Guidelines* is an accepted standard and can be accessed electronically at: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

Microorganisms that are RG1 require standard laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities. Many of the agents likely to be handled experimentally at Caltech are RG1 or RG2 pathogens, designated as low and moderate hazards, respectively. RG2 agents typically require slightly more sophisticated engineering controls (e.g., facilities and equipment) than standard laboratories, as well as special handling and decontamination procedures.

A number of RG2 agents have been associated with laboratory-acquired infections (LAI). The progression from invasion - to infection - to disease following contact with an infectious agent depends upon the route of transmission, inoculum, invasive characteristics of the agent, and resistance of the person exposed (whether innate or acquired). Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. It is important to always assume virulence and handle such agents at the prescribed biosafety level.

B. ROUTE OF INFECTION

Depending on the organism, pathogens are transmitted via several possible routes of infection. The most common routes of infection are inhalation of infectious aerosols, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, or percutaneous inoculation (injection, incision, or animal scratch or bite). Appropriate precautions should be implemented to reduce the risk of such exposures.

C. EXPOSURE SOURCES

HUMAN PATHOGENS

Microorganisms, e.g. fungi, bacteria, virus, parasites, rickettsia, prions, classified at RG2 and above are capable of causing infection and disease in humans.

PLANT AND ANIMAL PATHOGENS

Plant and animal pathogens may be classified as RG1 and could be handled at BSL1 or BSL2. A USDA/APHIS permit is required for the use of plant pathogens regardless of the biosafety level. Contact the Safety Office for help in completing the permit application form. All plant and animal pathogens classified as RG2 must be registered with the IBC prior to be handled in the laboratory.

RG2 organisms are not the only biohazard exposure sources in research laboratories. When performing the laboratories biohazardous risk assessment, one should also consider the following:

CLINICAL AND PATHOLOGICAL SPECIMENS

Any specimen from human patients or animals may contain infectious agents. Specimens most likely to harbor such microorganisms include blood, sputum, urine, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, feces, and tissues. Personnel in laboratories handling human blood, body fluids, non-human primate material, or even human cell lines that have been screened for pathogens should practice *universal precautions,* an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), Hepatitis C (HCV) and other bloodborne pathogens.

Biosafety Level 2 practices and containment must be followed when handling human materials that may contain bloodborne pathogens, e.g. HBV, HCV and HIV. The OSHA Bloodborne Pathogens Standard (California Code of Regulations, Title 8, Section 5139) applies to all occupational exposure to human blood or other potentially infectious human materials. Under the OSHA BBP standard, Caltech is required to develop and maintain a written Exposure Control Plan, offer employees the hepatitis B vaccination when applicable, provide annual training and post-exposure medical evaluation. For more information on the OSHA BBP standard see the Caltech Exposure Control Plan.

Animals may harbor endogenous pathogens that are virulent for humans. For personnel handling these animals or their tissues/body fluids, we recommend an analogous approach to infection control, *universal precautions,* which assumes these animals and their blood and body fluids to be infectious.

Non-human primate unfixed tissue and cell culture pose special risks as many of their diseases are often transmissible to humans and can be a serious health hazard. **Unfixed tissue and cells from macaques can carry the herpes-B virus that can be fatal in humans.**

CULTURES

BSL2 practices should be used for cell lines of human origin, even well-established lines such as HeLa and HEK293, and for all human clinical material (e.g., tissues and fluids obtained from surgery, autopsy or harvested on donors). Non-human primate cell cultures derived from lymphoid or tumor tissue, cell lines exposed to or transformed by a non-human primate oncogenic virus, and all non-human primate tissue should also be handled at BSL2.

OSHA considers both primary and established human cell lines to potentially contain bloodborne pathogens. Therefore laboratory personnel handling cell cultures are required by Federal law (California Code of Regulations, Title 8, Section 5139) to undergo Bloodborne Pathogens (BBP) training. At Caltech, this training requirement can be satisfied by attending an in-person training session. For information on obtaining this training, go to http://www.safety.caltech.edu/training/bbp_medwastemgmt.

When a cell culture is inoculated with (or known to contain) an etiologic agent with higher biosafety level, it should be classified and handled at the same biosafety level as the agent.

ANIMALS

Exercise care and thoughtfulness when using animals to isolate and/or propagate microorganisms, study pathology, or produce antibodies. Laboratory animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted material. In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols. During surgical procedures, necropsies, and processing of tissues, aerosols can be produced unintentionally, or the operator can inflict self-injury with contaminated instruments. Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols when changing bedding and cleaning cages.

D. HEALTH STATUS

Some unusual circumstances warrant special considerations or measures to prevent infection of laboratory personnel by certain microorganisms.

Regardless of the risk group of the organism you work with, it is good practice to inform your personal physician about your occupational risks, especially work with biohazardous or potentially biohazardous agents, so he or she may have a record of this information. Certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include, but are not limited to: diabetes or other metabolism disorders, pregnancy, certain autoimmune diseases, immunodeficiency or immunosuppression, animal-related allergies, chronic skin conditions or respiratory disorders, and steroid therapy, even if only temporary.

CHAPTER IV: BIOHAZARD CONTAINMENT

Although the most important aspect of biohazard control is the awareness and care used by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can prevent dissemination of pathogens should their accidental release occur.

A. BIOSAFETY LEVELS

Biosafety Levels consist of combinations of laboratory practices and procedures, safety equipment, and laboratory facility design features appropriate for the operations to be performed within the lab, and are based on the potential hazards imposed by the agents used and for the specific lab activity. **It is the combination of practice, equipment, and facility design that form the basis for physical containment strategies for infectious agents.**

There are four biosafety levels, with Biosafety Level 1 (BSL1) being the least stringent and Biosafety Level 4 (BSL4) being the most stringent. In general, BSL1 is recommended for work with nonpathogenic microorganisms, BSL2 is recommended for disease agents transmitted by direct contact (percutaneous inoculation, ingestion, or mucous membrane exposure), BSL3 is recommended for disease agents with a higher potential for aerosol transmission, and BSL4 is recommended when total separation between the infectious agent and investigator is critical.

Risk Group designations **often, but not always**, correlate directly with the biosafety level appropriate for a given research activity. For example, deleting the virulence factor of a RG3 pathogen may render it safe to be handled with BSL2 facility and practices. Conversely, insertion of toxin-producing genes in an RG1 microorganism may require BSL2 facility and practices. Furthermore, RG2 agents with high potential of causing mutagenesis may require additional BSL3 practices in a standard BSL2 facility.

The Institutional Biosafety Committee (IBC), established under the NIH Guidelines, determines the proper biosafety level for working with a particular project. One should always carefully review project-specific, approved IBC protocol prior to starting the research. This manual is designed to focus on BSL2, but a brief description of the Biosafety Level and the facility design features appropriate for labs operating at the various biosafety levels is presented in the following table.

SUMMARY OF BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

Source: BMBL, 5th Edition

B S L	LABORATORY PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (secondary barriers)
1	Standard Microbiological Practices	None required: open bench work PPE should be considered for chemical hazards	Hand washing sink
2	**BSL1 practice plus**: • Access control • Biohazard signage • Universal precautions for handling sharps • Biosafety Manual defining any needed waste decontamination or medical surveillance	**Primary barriers**: • Class I or II biosafety cabinet or other physical containment devices used for manipulations of agents with high potential of splashes or aerosols of infectious materials • **Personal Protective Equipment**: Laboratory coats; gloves; face protection as needed	**BSL1 plus**: Recommended directional air flow • Eye wash
3	**BSL2 practice plus**: • Secured access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum (if applicable)	**Primary barriers**: • Class I or II biosafety cabinet or other physical containment devices used for all open manipulation of agents • **Personal Protective Equipment**: Protective laboratory clothing; gloves or double gloves; respiratory protection as needed	**BSL2 plus**: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated and HEPA filtered • Negative airflow into lab required
4	**BSL3 practices plus**: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	**Primary barriers**: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	**BSL3 plus**: • Separated building or isolated work zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements

The BMBL also describes animal biosafety levels for the use of research animals. For more detailed information on biosafety levels, go to the CDC website: http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_IV.pdf

Most Caltech Laboratories operate at either BSL1 or BSL2. A summary of the biosafety levels from the BMBL is provided below.

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. **Work is typically conducted on open bench tops using standard microbiological practices.** Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessments.

Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. Lab coat, gloves, and eye protection are not required but recommended at BSL1. Nonetheless, PPE should be considered at BSL1 upon performing a risk assessment about the chemical hazards present and used in the lab.

Biosafety Level 2 builds upon BSL1. BSL2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. **With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low.** Hepatitis B virus, *Salmonella* and *Toxoplasma,* are representative of microorganisms handled at this containment level. BSL2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human- derived materials should refer to the OSHA Bloodborne Pathogens Standard for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers, such as hand washing sinks and waste decontamination procedures, must be available to reduce potential environmental contamination.

B. PRACTICES AND PROCEDURES

The following practices, **corresponding to BSL2,** are important for the prevention of laboratory infection and disease, as well as for the reduction of the potential for contamination of experimental material. These practices and procedures provide the foundation for the more restrictive containment of RG3 organisms. If you are considering research with a RG3 organism, contact the Safety Office at x 6727 for additional information.

PERSONAL HYGIENE

a) Do not eat, drink, chew gum, use tobacco, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
b) Do not store food for human consumption in laboratory refrigerators.
c) Wash hands frequently after handling infectious materials, after removing latex/nitrile gloves and protective clothing, and always before leaving the laboratory.
d) Keep hands away from your mouth, nose, eyes, face, and hair.

e) Do not remove personal protective equipment (such as cloth lab coats) from the lab.
f) First-aid kit(s) should be available, fully stocked and current (not expired).

HAND HYGIENE

Hand hygiene prevents infection and the spread of contamination, and it is the responsibility of all individuals working with biohazardous agents and laboratory animals to practice proper hand hygiene. Hand hygiene should be performed:

- After contact with potentially infectious material on gloved or bare hands;
- Before and after contact with lab animals;
- After removing gloves; and
- Before eating

Most transient bacteria present on the hands are removed during the mechanical action of washing, rinsing and drying hands. Hand washing with soap and running water must be performed when hands are visibly soiled. If running water is not available, use moistened wipes to remove all visible dirt and debris, followed by an alcohol-based hand rub.

HAND WASHING TECHNIQUE

Source: Centers for Disease Control Website

- **Wet** your hands with clean, running water (warm or cold), turn off the tap, and apply soap.
- **Lather** your hands by rubbing them together with the soap. Be sure to lather the backs of your hands, between your fingers, and under your nails.
- **Scrub** your hands for at least 20 seconds. Need a timer? Hum the "Happy Birthday" song from beginning to end twice.
- **Rinse** your hands well under clean, running water.
- **Dry** your hands using a clean towel or air dry them.

USING HAND SANITIZER

Source: Centers for Disease Control Website

Washing hands with soap and water is the best way to reduce the number of microbes on them in most situations. If soap and water are not available, use an alcohol-based hand sanitizer that contains at least 60% alcohol. Alcohol-based hand sanitizers can quickly reduce the number of microbes on hands in some situations, **but sanitizers do not eliminate all types of germs.**

Although alcohol-based hand sanitizers can inactivate many types of microbes very effectively when used correctly, people may not use a large enough volume of the sanitizers or may wipe it off before it has dried. Furthermore, soap and water are more effective than hand sanitizers at removing or inactivating certain kinds of germs, like *Cryptosporidium*, norovirus, AAV and *Clostridium difficile*. Hand sanitizers are not as effective when hands are visibly dirty or greasy.

How to use the hand sanitizer:

- Apply the product to the palm of one hand (read the label to learn the correct amount).
- Rub your hands together.
- Rub the product over all surfaces of your hands and fingers until your hands are dry.

LABORATORY PROCEDURES FOR HANDLING INFECTIOUS MICROORGANISMS

a) This Biosafety Manual outlines general activities and defines standard operating procedures (SOP). In most cases, your lab's Institutional Biosafety Committee (IBC) protocol, together with this Biosafety Manual, will provide you with the necessary information to work safely. However, the IBC may require laboratories to provide a dedicated Laboratory Biosafety Manual to outline specific laboratory activities and define custom laboratory SOPs.
b) If you are working with recombinant DNA and/or working with biological material at BSL2 or higher, you must obtain approval by the Caltech IBC. The IBC Administrator can be reached at x4699 or online at: ibc@caltech.edu.
c) Principal Investigators and/or laboratory supervisors are responsible for training employees and ensuring that all personnel are informed of specific hazards.
d) Plan and organize materials/equipment before starting work.
e) Keep laboratory doors closed; limit access to lab personnel.
f) When RG2 (or higher) pathogens are used in long-term studies, post a biohazard sign at the laboratory/room entrance identifying the agents in use and the appropriate emergency contact personnel. Templates of these biohazard signs will be generated by the Safety Office based upon the information provided in your lab's IBC Protocol. The Safety Office can be reached at x6727 or online at safety@caltech.edu
g) BSL2 laboratories should have a sink for hand washing, an eyewash station in which the eyewash is tested/flushed monthly, be relatively clutter-free, and be easy to access.
h) Wear a fully fastened laboratory coat when working with infectious agents. Wear protective gloves whenever handling potentially hazardous materials, including human blood and body fluids. Wear eye protection when working in the BSL2 laboratory when necessary.
i) Remove and leave all protective clothing, including gloves, within the laboratory before exiting. If transport of research materials through public spaces is required, one glove may be removed and ungloved hand used to handle public equipment (door handles, elevator buttons, etc.) and lab coats may be carried.
j) Never mouth-pipette; use mechanical pipetting devices.
k) When practical, perform all aerosol-producing procedures such as shaking, grinding, sonicating, mixing, and blending in a properly operating biological safety cabinet (BSC). Note that placement of certain equipment within the BSC may compromise cabinet function by disturbing the air curtain. BSC certification and annual re-certification should be performed with permanent equipment inside the BSC.
l) Centrifuge materials containing infectious agents in durable, shatter-resistant, closable tubes. Use a centrifuge with sealed heads or screw-capped safety cups. After centrifugation, open the tubes within a BSC.
m) Minimize the use of needles, syringes, razor blades, and other sharps when possible. After use, syringe-needle units must be disposed in a dedicated sharps container without removing or recapping the needles.
n) Cover countertops where hazardous materials are used with plastic-backed disposable paper to absorb spills and dispose of the paper daily or following a spill.
o) Wipe work surfaces with an appropriate disinfectant according to the corresponding IBC protocol after experiments and immediately after spills.
p) Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal.
q) Report all accidents, spills, and near misses to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol and the location of cleanup equipment. Step-by-step Spill response protocols should be posted in the laboratory.

r) Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Do not forget to routinely decontaminate all shared equipment and equipment in common areas.
s) Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate biohazard signs.
t) Equipment used with biohazards must be decontaminated prior to repair.
u) It is recommended to never work alone in a laboratory while handling hazardous material of any type or when engaging in any dangerous activities.

CONSIDERATIONS FOR BIO-AEROSOLS

When manipulations of microorganisms or cell cultures present a potential to create aerosols, use a biological safety cabinet (BSC). Do not use a clean bench as it will not protect you from potential exposure to pathogens. Conversely, a fume hood will protect you but will not protect your sample from contaminants in the ambient air.

Accidental spilling of infectious liquid cultures is an obvious hazard due to the generation of aerosols and/or small droplets. However, even routine manipulations of cultures may release microorganisms via aerosol formation:

EXAMPLE OF PROCEDURES THAT GENERATE AEROSOLS:

- Popping stoppers from culture vessels.
- Opening closed vessels after vigorous shaking.
- Spattering from flame-sterilized utensils.
- Expelling the final drop from a pipette.
- Spinning microfuge tubes in a standard microfuge.
- Vortexing liquid samples.

WHAT TO DO TO LIMIT AEROSOLS GENERATION/DISSEMINATION:

- Manipulate cultures of infectious material carefully to avoid the uncontrolled release of aerosols or the generation of large droplets or spills.
- Centrifuge cultures using gasket-sealable tubes, carriers, and rotors, when available.
- Seal microplate lids with tape or replace them with adhesive-backed Mylar film.
- When vortexing infectious samples, ensure there is a tight seal.
- Load, remove, and open tubes, plates, and rotors within a biological safety cabinet or fume hood.

SHARPS PRECAUTIONS AND DISPOSAL

NEVER USE 2 HANDS TO RECAP NEEDLES!

Other Needle precautions include:

- Avoid the use of needles and other sharps whenever possible. Many glass items have plastic alternatives that could be used.
- If the use of sharps is unavoidable, take extra precautions.
- **Dispose of needles immediately after use in a red biohazardous sharps container.**
- Never overfill biohazardous sharps containers. When the container is 2/3 full as indicated by the “full line” on the container, close it, and contact the EHS office for pick up by completing the online request form via the AiM portal.
- Use syringes with a luer-lock system to prevent the needle from detaching from the syringe during use.
- Never recap needles using two hands. If a needle must be recapped, use a one-handed method or a

mechanical device, e.g. forceps or hemostats.

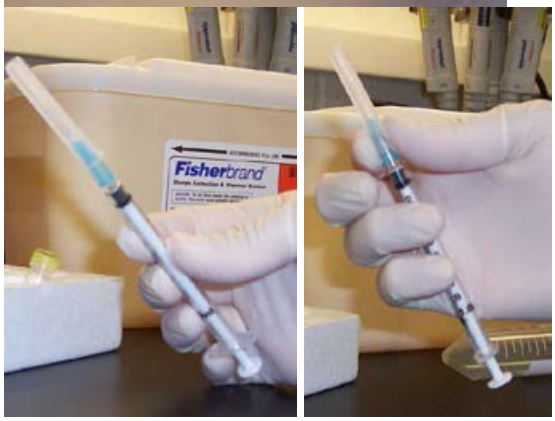
- Never remove needles from the syringe and never shear, bend or break needles.
- Never pass uncapped needles directly to another person.
- Never remove needle caps with your mouth.
- If using needles to inject animals, always restrain the animal to prevent inadvertent movement.
- If using needles to inject small lab animals, e.g. intravenously or intramuscularly, always anesthetize the animal per OLAR requirements.
- Always use a mechanical device to remove scalpel blades, never use your fingers.
- Contact the EHS Office for help in evaluating or selecting safer medical devices, e.g. safe needles.

ONE-HANDED NEEDLE RECAPPING AND NEEDLE HOLDING DEVICES

Although recapping needles is not recommended in the lab, there are times in which it must be done. In the event that needles must be filled in advance of their use, there are safe methods that can be used to "recap" them using one hand. Here are suggestions for doing this in a safe manner:

"One-handed scoop" method:

Place the cap on the bench top and hold the syringe in one hand. Keep the other hand by your side. Slide the needle into the cap, then lift it up and snap it on securely using only one hand.



Using a sterile 50 mL centrifuge tube or Styrofoam rack:
Place the uncapped needle inside a conical tube temporarily instead of recapping. Alternatively, put the cap inside an open centrifuge tube or rack so that the needle can be inserted into it and the cap and secured by firmly pushing the needle downward into it. There are also commercial needle recapping devices available for this purpose.

Remember to keep a designated sharps container nearby for disposal of sharps, and don't recap unless absolutely necessary.

C. ENGINEERING CONTROLS

LABORATORY DESIGN

The more virulent an organism, the greater the degree of physical containment required. Proper safety equipment provides primary containment; laboratory design provides secondary containment. The Institute Biosafety Officer is available for consultation on these matters.

LABORATORY VENTILLATION (HVAC)

- **To control containment it is important that laboratory air pressure be lower than that in the adjacent spaces. This negative air pressure differential ensures that air will enter the laboratory and not egress to the hallway. While negative air pressure is recommended at BSL2, it is required at BSL3.**
- **If you wish to maintain negative room pressure, laboratory doors should be kept closed while biohazardous work is taking place.**

BIOSAFETY CABINETS (BSC)

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. When functioning correctly and used in conjunction with good microbiological techniques, BSCs are very effective at controlling infectious aerosols. BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed.

BSCs control airborne contaminants during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. The Class II Biosafety Cabinet (BSC) is the most commonly used BSC at Caltech. It is designed to protect the user, the product and the environment from infectious materials inside the cabinet and to protect the material inside the cabinet from contamination from the lab environment.

BSC TYPES

Three kinds of biological safety cabinets, designated as Class I, II, and III, have been developed to meet varying research and clinical needs.

CLASS I cabinets are manufactured on a limited basis and have largely been replaced by Class II cabinets. A Class I cabinet is essentially a HEPA-filtered chemical fume hood in which all of the air entering the cabinet is exhausted into the room or ducted to the outside.

CLASS II – The most utilized class of BSC on campus. Two varieties of Class II BSCs are used and both are adequate for manipulations of RG2 or RG3 pathogens.

- CLASS II TYPE A—recirculates 70% of the internal air and exhausts 30% of filtered air into the laboratory.

- CLASS II TYPE B—either recirculates 30% of internal air and exhausts 70% of filtered air through a duct to the outside atmosphere or has 100% total exhaust cabinets. **Because of the greater safety margin, small amounts of nonvolatile chemical, carcinogens, or radioactive materials can be used in this cabinet.**

BIOSAFETY CABINET LOCATION AND CERTIFICATION

Since the air curtain created at the front of the cabinet can be easily disrupted, a biosafety cabinet should be located away from air supply registers, entrances, high traffic areas, and laboratory equipment, e.g. centrifuges, which create turbulence.

- A biosafety cabinet used with biohazardous materials must be professionally certified after installation, annually, and after being moved.
- The company chosen to perform the certification should be able to provide proof of NSF/ANSI 49 accreditation for each field technician sent to service BSCs at Caltech.
- A biosafety cabinet must be properly decontaminated before being moved.
- It is the Laboratory/Department responsibility to contact their preferred BSC certification vendor for these services.

Horizontal laminar flow clean benches are not biological safety cabinets and should never be used for work

with potentially hazardous materials, whether biological or chemical. These devices protect the material in the cabinet but not the worker or the environment. Similarly, chemical fume hoods are not biological safety cabinets. They draw air in, potentially protecting the worker, but do not protect the material in the cabinet (your samples), and exhaust aerosolized material and vapors/gases into the environment.

Many BSCs have ultraviolet lamps inside them. These lamps provide only limited ability to inactivate microbes. Efficacy is limited to exposed surfaces and penetration of organic material is very poor. Note that effectiveness decreases as the lamp ages. Furthermore, exposure to ultraviolet light may cause eye damage.

Therefore, ultraviolet lamps are not recommended to be the sole source of decontamination of BSC surfaces.

SAFE AND EFFECTIVE USE OF A BIOSAFETY CABINET

- **START UP**
  - Monitor alarms, pressure gauges, or flow indicators for any changes. Do not work in a biosafety cabinet while a warning light or alarm is signaling.
  - Shut off the UV light.
  - Turn on blower and fluorescent light.
  - Wait at least two minutes before loading equipment. This is to purge the BSC of contaminated air.
  - Check grilles for obstructions.
  - Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
  - Adjust the sash to proper position; NEVER use above the 8-inch mark.
  - Restrict traffic in the BSC vicinity. To ensure proper functioning of a BSC, it is best to locate them away from high-traffic areas and doorways to common areas.
  - Plan your work and place everything needed for the procedure inside the BSC.
- **LOADING MATERIALS AND EQUIPMENT**
  - Load only items needed for the procedure.
  - Do not block the rear or front exhaust grilles.
  - Arrange materials to minimize movement within the cabinet.
  - Arrange materials within the cabinet from CLEAN to DIRTY (or STERILE to CONTAMINATED).
  - Materials should be placed at least six inches from the front BSC grille.
  - Never place non-sterile items upstream of sterile items.
  - Maintain the BSC sash at proper operating height, approximately level with your armpits.
- **RECOMMENDED WORK TECHNIQUE**
  - Wash hands thoroughly with soap and water before and after any procedure.
  - Wear gloves and lab coat/gown; use aseptic techniques.
  - Avoid blocking front and back grilles. Work only on a solid, flat surface; ensure chair is adjusted so armpits are at elevation of lower window edge.
  - Avoid rapid movement during procedures, particularly within the BSC, but in the vicinity of the BSC, as well.
  - Move hands and arms straight into and out of work area; never rotate hand/arm out of work area during a procedure.
  - Two people working together in one BSC is not recommended; however in the event it is necessary ensure that both workers are following the correct precautions.
  - Place a centrifuge or blender that creates air turbulence in the back 1/3 of the cabinet and stop other work while the equipment is running.
  - **Don't operate a Bunsen burner in the cabinet.** Open flames are not required in the clean environment of a BSC. An open-flame creates turbulence that disrupts the pattern of HEPA-filtered supplied air to the work area. When necessary a touch-plate microburner can be used to supply a flame on demand that will minimize air disturbance and heat build-up. Use disposable sterile loops or an electric bacti-incinerator to sterilize bacteriological loops.
- **FINAL PURGING AND WIPE-DOWN**
  - After completing work, run the BSC blower for two minutes before unloading materials from the cabinet.
  - Disinfect the exterior of all containers BEFORE removal from the BSC.
  - Decontaminate interior work surfaces of the BSC with an appropriate disinfectant.

- **DECONTAMINATION AND SPILLS**
  - All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. The final surface decontamination of the cabinet should include a wipe-down of the entire work surface. Investigators should remove their gloves and gowns or lab coat, and wash their hands as the final step in safe microbiological practices.
  - Small spills within the BSC can be handled immediately by covering the spill with absorbent paper towels, carefully pouring an appropriate disinfectant onto the towel-covered spill, and removing the contaminated absorbent paper towels and placing them into the biohazard waste bag. Any splatter onto items within the cabinet, as well as the walls of the cabinet interior, should be immediately wiped with a paper towel dampened with disinfectant. Gloves should be changed after the work surface is decontaminated. Hands should be washed whenever gloves are changed or removed.
  - Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution (10% bleach) can be poured onto the work surface and through the grille(s) into the drain pan. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant (nature and concentration) and the microbiological agent. The drain pan should be emptied into a collection vessel containing disinfectant. Drain pan should be wiped down with 70% alcohol to prevent corrosion.
- **MAINTENANCE**
  - To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be certified and leak tested. Caltech requires that all BSCs be certified annually by an outside, certified and NSF/ANSI-49 accredited company. **This is imperative for BSCs intended for work at BSL2**.

AEROSOL-PROOF ROTORS AND SAFETY CUPS FOR CENTRIFUGES

Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes breaking. Procedures for centrifuging biohazardous materials include:

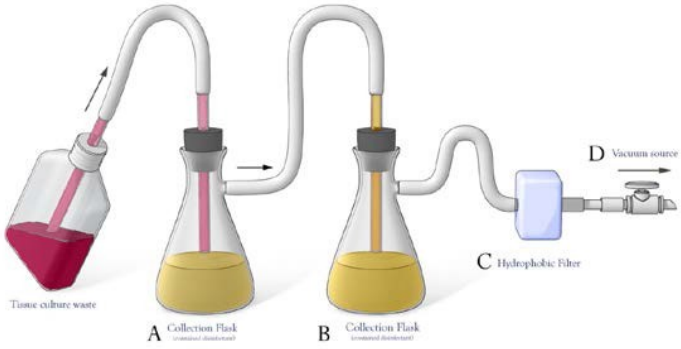
- Use aerosol-proof rotors or safety buckets with caps that seal with intact O-rings.
- Before use, inspect O-rings and safety caps for cracks, chips, or erosion.
- Use tubes with threaded caps as much as possible.
- Never overfill centrifuge tubes since leakage may occur. Fill tubes no more than ¾ full and avoid getting caps/closures wet.
- Wipe tubes down with a disinfectant after filling.
- **Load and unload rotors and buckets inside the BSC.**
- Balance buckets, tubes and rotors before centrifuging.
- **Periodically disinfect the centrifuge.**
- Small, low-speed centrifuges may be placed in the back 1/3 of the BSC to contain aerosols.

OTHER SAFETY EQUIPMENT FOR AROSOL-PRODUCING DEVICES

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

Safety blenders and the BeadBeater homogenizer (BioSpec) are designed to prevent leakage of aerosols.

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



VACUUM LINE PROTECTION

To protect vacuum lines, aspiration flasks used with biohazardous materials should have an overflow flask attached to the collection flask. In addition, a hydrophobic vacuum line filter should be used if aspirating biohazardous material. Liquids traps should also be secured in leak proof secondary containers to avoid leak age on the lab floor.

D. PERSONAL PROTECTIVE EQUIPMENT (PPE)

Personal protective equipment (PPE) is used to protect personnel from contact with biohazardous materials. PPE use is designed to reduce the risk of contamination of personal clothing, reduce exposure of skin and mucous membranes to biohazardous material, and reduce transmission of pathogens outside of the laboratory area.

Personal protective equipment is provided to employees at no cost to them. The type of PPE used will depend on the procedures being performed. For assistance in selecting PPE, contact the Safety Office online at safety@caltech.edu or by calling x6727. The following PPE should be made available in laboratories operating at BSL2.

LABORATORY COATS

Lab coats must be worn when there is a risk of biohazardous material contacting a worker's skin or clothing.

- Lab coats are meant to protect clothing from contamination. Cloth lab coats are not fluid resistant in situations where splashing or soaking with biohazardous materials is anticipated. Fluid-resistant lab coats must be used in situations where soaking or splashing may occur.
- Lab coats should be changed promptly whenever they become visibly soiled or contaminated with biohazardous material.
- Lab coats may be disposable or reusable. Reusable lab coats must be laundered on site or by a commercial laundry service that has procedures in place for safe handling of garments that may have been exposed to biohazards. **Laboratory coats may not be taken home for laundering**. Disposable lab coats that may have contacted biohazardous material must be discarded in red biohazardous waste bags.
- Lab coats must not be taken home or worn in non-laboratory areas, e.g. restrooms and cafeterias.
- When required, lab coats should be provided for visitors as well as maintenance and service workers.

GLOVES

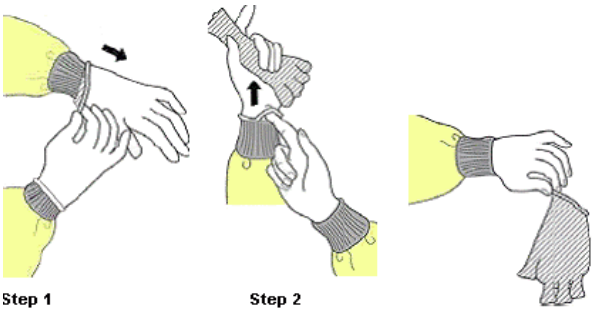
Gloves must be worn to protect hands from exposure to biohazardous materials. Gloves are worn when there is a possibility of direct contact with biohazardous material. Cuts or lesions on the hands or arms should be bandaged before donning gloves. The selection of gloves should be based on the type of procedure being performed.
Disposable latex or nitrile gloves are designed to fit the hand tightly to allow for the performance of delicate manipulations. If an employee has a skin reaction from the gloves, hypo-allergenic and/or powder-free gloves must be provided.

All employees using gloves must observe the following precautions:

- **Disposable gloves should not be re-used. Never wash / decontaminate disposable gloves for re-use.**
- Replace gloves if torn, punctured, or contaminated.
- Avoid using gloved hands to touch surfaces that will be touched by people with non-gloved hands.
- Avoid contamination of personal items such as telephones, pens, and electronic devices. Telephones should never be answered while wearing gloves.
- Wash hands or use an alcohol-based hand sanitizer immediately after glove removal. Using disposable gloves does not negate the need for hand hygiene. **Gloves do not provide complete protection against hand contamination, thus performing hand hygiene immediately after removing gloves is essential.**
- Dispose of used gloves with other contaminated laboratory waste.
- Remove gloves in such a way to avoid contact with the exterior of the glove, as pictured below.

Proper Glove Removal

Source: Centers for Disease Control and Prevention Website



Step 1: Grasp the exterior of one glove with your other gloved hand. Carefully pull the glove off your hand, turning it inside-out. The contamination is now on the inside. Ball the glove up and hold in your other gloved hand.

Step 2. Slide your ungloved finger into the opening of the other glove. Avoid touching the exterior. Carefully pull the glove off your hand, turning it inside out again. All contamination is contained.

EYE PROTECTION AND FACE MASKS

Face protection, e.g., safety glasses, goggles, face shield, or surgical face mask, must be worn when there is a risk of splashing biohazardous material. Face masks must be worn so that they completely cover the nose and mouth and fit closely to the face. When using a face mask, the blue side should face out, and the metal nose-band must be shaped to the face.

Eye protection equipment should be decontaminated or discarded and replaced as often as necessary. Disposable masks must be removed immediately after use and not re-used. Always discard disposable protection equipment in the biohazardous waste bin.

RESPIRATORY PROTECTION

Respiratory protection is designed to protect personnel from biohazardous agents transmitted by the aerosol route. An N95 respirator is a disposable HEPA filter respirator. Personnel must complete a respirator medical questionnaire, be medically cleared by a doctor, and be fit-tested before using an N95 respirator. Contact the Safety Office for more information.

FOOTWEAR AND DRESS CODE

Closed toed footwear must be worn to reduce the risk of injury from dropped equipment and to protect the feet from contact with potentially infectious materials. Neither shorts nor skirts are permitted in BSL2 laboratories.

E. TRAINING

Laboratory safety begins with a comprehensive assessment of risks posed by research reagents and associated lab activities as well as an assessment of compliance issues associated with research conducted within that lab. An important component of this risk assessment process is the identification of laboratory safety issues that can only be mitigated through appropriate training. A comprehensive laboratory safety training program involves specialized training elements (Biosafety Courses offered by the Institute Biosafety Officer) as well as lab or project-specific training elements provided by each PI. The final determination of which of the available Biosafety Courses (listed below) is/are required for personnel associated with a given protocol is made by the IBC during their review of the IBC Protocol submission. The training programs currently offered by the BSO and when they are required are described below.

INSTITUTIONAL BIOSAFETY TRAINING PROGRAM

BSL1 Course This training module is required for all personnel listed on an IBC protocol that describes work managed at BSL1 utilizing recombinant or synthetic nucleic acids (as defined in the *NIH Guidelines*). Each individual shall complete this course every three (3) years unless a significant change in legal or Institutional policy or safety guidelines dictate a shorter time interval.

BSL2 Course: This training module is required for all personnel listed on an IBC protocol that describes work managed at BSL2 with or without the usage of recombinant or synthetic nucleic acids. This includes work with biological toxins regulated by the IBC. If a protocol includes both BSL2 and BSL1 work, the rDNA/BSL2 Course will satisfy both requirements. Each individual shall complete this course every three (3) years unless a significant change in legal or Institutional policy or safety guidelines dictate a shorter time interval.

Viral Vectors Course: This training module is required for all personnel listed on an IBC protocol that describes work utilizing viral vectors (both replication competent and incompetent) regardless of the biosafety level used to manage them. Each individual shall complete this course every three (3) years unless a significant change in legal or institutional policy or safety guidelines dictate a shorter time interval.

Biological Toxins Course: This training module is required for all personnel listed on an IBC protocol that describes work with any of the toxins regulated by the IBC. Each individual shall complete this course every three (3) years unless a significant change in legal or Institutional policy or safety guidelines dictate a shorter time interval.

OSHA Bloodborne Pathogens Training: This training is required for all personnel listed on an IBC protocol that describes work with human cells, blood, or tissues. Each individual shall complete this course annually as stated in the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (California Code of Regulations, Title 8, Section 5139). Initial and annual training provided by the Institute Biosafety Officer is documented in the OLM database.

LABORATORY-SPECIFIC OR JOB-SPECIFIC TRAINING

As a supplement to the Institutional Biosafety training, the PI or his/her designee must provide laboratory-specific training to new personnel on the location of emergency equipment and emergency response guides, type of experiments being conducted, the nature of the material and equipment used and their associated hazards, safe work practices, waste management, and dealing with accidents including reporting requirements. The laboratory should maintain the laboratory-specific training documentation.

F. MEDICAL SURVEILLANCE

Caltech contracts with two commercial occupational health clinics, Pasadena Community Urgent Care (PCUC) and St. George's Medical Clinic, to provide occupational health services for personnel who are occupationally at risk of exposure to bloodborne pathogens (BBP) and other biohazardous agents. Any individual who receives an exposure or potential exposure will be given a medical consultation and advised of available treatments.

In addition to post-exposure evaluation and follow up, the program includes free vaccination to employees who are occupationally at risk of exposure to agents for which a vaccine is available including, but not limited to hepatitis B virus vaccine, the pneumococcal vaccine, and the SAD rabies vaccine.

LABORATORY ANIMAL USERS OCCUPATIONAL HEALTH PROGRAM

Caltech is required by the Public Health Service Policy to provide an occupational health and safety program for personnel working with laboratory animals. The program is designed to protect the health of people that have direct contact with laboratory animals and personnel, e.g. custodians and maintenance workers, who may work in laboratory animal areas. The program includes vaccination offers as needed, pre-screening and annual medical history questionnaires, risk assessment forms, respiratory protection medical questionnaires, as needed, and post-exposure medical treatment. The Risk Assessment and Medical/Respiratory Questionnaire Forms are submitted online directly to the Occupational Health physician. Please access the online tool here:
http://www.safety.caltech.edu/manuals/form_laohp_risk_assess

The IACUC Coordinator notifies the research staff to submit their medical questionnaires to the Occupational Health Clinic. IACUC SOP # O-53 describes the Institute Animal Care and Use Committee (IACUC) policy for participation in the laboratory animal occupational health program.

CHAPTER V: BIOLOGICAL WASTE DISPOSAL PROCEDURES

Remember: "*It is not because your experiment is done, that the risk is gone*"
Dr. L. Quenee, Institute Biosafety Officer

These biohazardous waste disposal guidelines are designed to not only protect the public and the environment, but also laboratory and custodial personnel, waste haulers, and landfill/incinerator operators at each stage of the waste-handling process. Generators of biohazardous waste in the laboratory must ensure that the labeling, packaging, and intermediate disposal of waste conforms to these guidelines.

"**Decontamination**" means a process of removing disease-producing microorganisms and rendering an object safe for handling.

"**Disinfection**" means a process that kills or destroys most disease-producing microorganisms, except spores.

"**Sterilization**" means a process by which all forms of microbial life, including spores, viruses, and fungi, are destroyed.

The California Department of Public Health regulates the disposal of biohazardous and medical waste per the Medical Waste Management Act, Health and Safety Code, Chapter 5 Section 117960.

DEFINITIONS

- **Biohazardous waste** is waste that may contain pathogens capable of replication and capable of causing disease in humans, animals, or plants.
- **Medical waste** is biohazardous waste and/or sharps waste that may contain agents infectious to humans.
- "**Not medical waste**", is defined in the Act as follows:
    - Waste generated in food processing or biotechnology that does not contain an agent infectious to humans.
    - Sharps waste that is not contaminated with medical waste.
    - Waste generated in biotechnology that does not contain human blood or blood products or animal blood or blood products suspected of being contaminated with agents infectious to humans.
    - Urine, feces, saliva, sputum, nasal secretions, sweat, tears, and vomitus, unless it contains fluid blood from humans or animals known or suspected to have agents that are infectious to humans.
    - Waste that is not biohazardous, including items such as paper towels, surgical gowns, or bandages that contain non-fluid blood.
    - Hazardous chemical waste, radioactive waste, and household waste.
    - Waste generated from normal and legal veterinary, agricultural, and livestock management practices.
- **Sharps container** is a rigid puncture-resistant container that, when sealed, is leak resistant and cannot be reopened without great difficulty.
- **Sharps waste** is any device having acute rigid corners, edges, or protuberances capable of cutting or piercing, including, but not limited to, all of the following:
    - Hypodermic needles, hypodermic needles with syringes, blades, needles with attached tubing, syringes contaminated with biohazardous waste, acupuncture needles, and root canal files;
    - Broken glass items, such as Pasteur pipettes and blood vials contaminated with biohazardous waste; and
    - Any item capable of cutting or piercing that is contaminated with trauma scene waste.

A. SOLID WASTE SEGREGATION, COLLECTION, CONTAINMENT, AND LABELING

SOLID BSL1 WASTE

Solid BSL1 waste that does not meet the definition of biohazardous waste should be segregated at the point of generation in each laboratory work area. The BSL1 waste is collected in clear autoclave bags that are **not** labeled as medical waste. These bags must **not** have biohazard symbols or any wording indicating medical waste, biohazard waste, or biohazard and should **not** be orange or red in color. When BSL1 waste contains recombinant DNA or

organisms or cells containing recombinant DNA, laboratory personnel are responsible for autoclaving this waste before disposal in the regular trash. Temperature indicator (autoclave tape w/ temperature sensitive strips) should be added in each waste load. If there is any indication that the autoclave run did not reach the proper temperature, time or if the temperature indicator did not turn black, the waste load should be re-treated prior to disposal.

SOLID BSL2 WASTE

Solid biohazardous and/or medical waste should be segregated at the point of generation in each laboratory work area.

- BSL2 waste is placed in **red biohazard bags** labeled with the biohazard symbol and the word "Biohazard."
- The red biohazard bags are contained in leak-proof hard-walled secondary containers with tight fitting covers. The secondary containers are labeled with the biohazard symbol and the word "Biohazard."
- Biohazard bags are tightly closed at the point of origin to prevent leakage or expulsion of contents when they are ready for transport, treatment, and disposal.
- Biohazard waste bags are not removed from the secondary container except for transfer to another secondary container or to the secondary storage container at the storage site.
- Bagged biohazardous waste is transported in tightly closed secondary containers to the designated storage site.
- Once a week a commercial waste hauler removes the biohazardous waste from the storage area for treatment at a licensed facility.

EHS-contracted personnel are responsible for transporting and storing BSL2 waste prior to off-site treatment. EHS-contracted personnel are also responsible for re-lining the red bins with new red bags upon waste pick-up from the lab. It is the labs' responsibility to ensure that red bins are available in the BSL2 laboratory and to ensure that EHS-contracted personnel have been informed of the need to come into the lab to pick-up the waste.

PLASTIC SEROLOGICAL PIPETTES AND PIPETTE TIPS - COLLECTION AND DISPOSAL

Plastic serological pipettes and plastic pipette tips cannot puncture skin and are not considered sharps. However, since these materials may poke through a biohazardous waste bag when collected with other solid biohazardous waste, e.g. flasks and tubes, they can be collected in an appropriate separate container if they are used with biohazardous materials. The following options are available:

- Biohazardous pipette tips may be collected within the biosafety cabinet in an old media bottle or other plastic receptacle. When the container is full, it is closed, and placed in the biohazardous waste bag.
- Plastic serological pipettes may be collected separately from other waste in the small step-can that EHS has determined has the appropriate dimensions to collect the long plastic serological pipettes in such a way to avoid sideways placement that may cause the pipette to poke through a bag. Thus, "bundling" the long plastic pipettes in this manner prevents the plastic pipettes from poking through the bags. Please contact the Institute Biosafety Officer for additional information at x6727.
- Alternatively, plastic tips and serological pipettes may be collected in red biohazardous sharps containers of the appropriate size and height. Avoid removing the lid, overfilling, or shaking/rearranging the contents of the red biohazardous sharps containers to accommodate these larger non-sharp items.

SHARPS WASTE COLLECTION PROCEDURES

Biohazardous sharps waste describes material used with biohazardous material that have sharp edges capable of causing punctures or cuts, including, but not limited to, the following: needles, scalpels, razor blades, slides, coverslips, Pasteur pipettes (thin glass pipettes), capillary tubes, and broken glass.

Biohazardous Sharps Collection:

- Collect biohazardous sharps in a rigid, red biohazardous sharps container that has the biohazard symbol. These are available in the VWR stockroom or on-line.
- To avoid injury, do NOT clip, bend, shear, or separate needles from syringes and do NOT recap needles unless a one-handed technique is used.
- Do not overfill the sharps container.
- When the container is 2/3 full, close it, and contact EHS for pick-up by completing the online request via the AiM portal.

Note: Sharps waste not used with BSL1 or BSL2 materials, other than needles and syringes, should be placed in sharps containers or other rigid puncture resistant, leak resistant container with a tight fitting lid, that are not labeled as medical waste. These containers should not have biohazard symbols or any wording indicating medical waste, biohazard waste, or biohazard material and should not be orange or red in color.

B. TREATING LIQUID WASTE BEFORE DRAIN DISPOSAL

Most fluid waste, including human blood or infectious cultures **that have been decontaminated** by the appropriate method, can be discarded by pouring into the sanitary sewer, followed by flushing with water. Care should be taken to avoid the generation of aerosols.

Procedures for bleach disinfection of BSL1 and BSL2 liquid waste including recombinant organisms prior to drain disposal:

- Recommended Personal Protective Equipment:
  - Lab coat
  - Latex or nitrile gloves
  - Safety glasses
- Approved Disinfectant
  - Bleach, a sodium hypochlorite solution (NaOCl), is a broad-spectrum disinfectant that is an effective disinfectant for enveloped viruses (e.g. HIV, HBV, HSV), vegetative bacteria (e.g. *Pseudomonas, Staphylococcus, and Salmonella*), fungi (e.g. *Candida*), mycobacterium (e.g. *M. tuberculosis* and *M. bovis*), and non-enveloped viruses (e.g. Adenovirus and Parvovirus).
- Concentration:
  - The appropriate concentration of sodium hypochlorite for disinfecting liquid BSL1 and BSL2 waste, e.g. supernatants from cell culture, is 5,000 ppm, approximately 0.5%. Household bleach is 5.2 - 6.1 % sodium hypochlorite, therefore a 1:10 (v/v) dilution of bleach to liquid biological waste is appropriate.
- Contact time:
  - An appropriate contact time of sodium hypochlorite with liquid waste is 20 minutes before drain disposal. After 20 minutes of contact, disinfected liquid waste is poured down the sink and the drain is flushed with water.
- Stability and Storage:
  - Bleach should be stored between 50 and 70°F. According to Clorox®, undiluted household bleach has a shelf life of one year from the date of manufacture, after which bleach degrades at a rate of 20% each year until totally degraded to salt and water. A 1:10 bleach solution has a shelf life of 24 hours.

Additional Information can be found in the bleach safety data sheet. See the EHS Safety Data Sheets.

USE BIOHAZARD WASTE (RED BAGS/RED CONTAINERS) AND PICK-UP SERVICES BY EHS FOR BSL2 WASTE DISPOSAL

DO NOT USE AUTOCLAVING AS A MEANS OF DISPOSAL FOR ANY BSL2 WASTE

C. MIXED WASTE DISPOSAL

Mixed hazardous or radioactive wastes are wastes that contain a mixture of two or more of the following: medical/biohazardous waste, radiological waste, and/or hazardous chemical waste.

Mixed waste requires special handling, treatment, and disposal. Please contact the EHS Office for proper disposal procedures prior to generating the mixed waste online at safety@caltech.edu or by calling x6727.

CHAPTER VI: DECONTAMINATION OF WORK SURFACES AND EQUIPMENT

Decontamination of surfaces and equipment or objects is achieved by disinfection or sterilization by which the material becomes safe for handling. Cleaning, such as the removal of organic matter, grime, and dirt, is an essential step in decontamination because the survival time of many infectious agents outside the host is prolonged by the presence of organic matter. Organic matter can also decrease the effectiveness of some disinfectants, e.g. wescodyne and bleach. In animal facilities where there is a large amount of organic matter, equipment must be cleaned before sterilization or disinfection. Some pathogens, e.g. clostridial spores and *Cryptosporidium*, are highly resistant to disinfection; therefore cleaning in these cases is particularly crucial in order to mechanically remove the organisms.

It is important to use a disinfectant that has been shown to be effective against the microorganism you are trying to destroy. The EPA registers chemical disinfectants, and these EPA-registered antimicrobial products may not make efficacy claims against specific pathogens unless the EPA has reviewed data to support the claim and approved the claim on the label.

Below is a list of common disinfectants used in the laboratory:

Disinfectant	Effective against	Dilution & Shelf life	Contact time	Properties	Use
Bleach	Bacterial spores, vegetative bacteria, most viruses, fungi	1:10 24 hrs diluted	10-15 min surfaces; 20 minutes for waste	Inactivated by organic matter, corrosive, eye, nose, respiratory irritant	Spills, waste before drain disposal
Ethanol	Broad spectrum of bacteria and viruses; **NOT effective against bacterial spores or non-enveloped virus**	70-85% stable	10 minutes	non-corrosive, no residue, flammable (NO drain disposal), eye irritant	Stainless steel surfaces, instruments
Accelerated hydrogen peroxide (Accel)	Broad spectrum of pathogens including norovirus and parvovirus	1:40 3 mos diluted	5 minutes	Non-flammable, less corrosive than bleach	Surfaces in animal facilities

Bleach Facts

- Useful for disinfection of waste before drain disposal.
- If used for surfaces, may need to be rinsed off with clean water after the contact time has elapsed to prevent corrosion on some surfaces.
- A 1:10 dilution of bleach has 5,000 ppm, or 0.5% available chlorine.
- Bleach degrades over time losing its effectiveness as a disinfectant.
  - According to Clorox, undiluted bleach degrades at a rate of 20% per year and a 1:10 bleach solution has a shelf life of 24 hours.
  - However, if diluted bleach is stored in tightly closed opaque bottles, it will retain activity for up to 30 days.
  - Undiluted bleach containers should be used within one year of receipt or used at higher concentrations to account for 20% degradation per year.
- Some commercially available bleach solutions, e.g., Bleach-rite (10% beach), contains a stabilizer that extends its shelf life up to 18 months.

Some disinfectants can be irritants. Ensure that all areas are well-ventilated during disinfection. Always apply the disinfectant according to the product instructions with attention to contact time and appropriate dilution. After disinfection, allow all surfaces to dry completely.

All surfaces where biohazardous agents were used should be chemically disinfected upon completion of procedures. Spills of biohazardous materials must be chemically disinfected immediately. Disinfect all equipment used with biohazardous materials before being moved or serviced.

CHAPTER VII: EMERGENCY PROCEDURES AND REPORTING

No matter how carefully one works, laboratory accidents occur and may necessitate emergency response. Emergency plans should be tailored for a given biohazardous situation. The laboratory supervisor should prepare instructions specifying immediate steps to be taken. These instructions should be displayed prominently in the laboratory and periodically reviewed with personnel. No single plan will apply to all situations but the following general principles should be considered:

A. INFECTIOUS AGENT SPILL RESPONSE

At Caltech, spills of potentially infectious materials shall immediately be contained and cleaned up by employees **properly trained and equipped** to work with potentially infectious materials. Ultimately, the goal of cleaning up any spill of infectious agent or potentially infectious agent is to ensure the safety of the researcher/student and those around him/her. When cleaning up a spill, there are several important points that all researchers/students should keep in mind:

- Many, but not all, pathogenic agents carry a risk of exposure by inhalation. Droplets are large and settle with gravity and can be easily cleaned. Aerosols are small and must be removed by the building's ventilation system. If the pathogen involved in the spill carries a risk of exposure via the aerosol route, *immediately* leave the area for 30 minutes to allow droplets to settle and aerosols to be removed.
- In order to ensure the safety of the researchers and anyone in the vicinity, it is important to contain the spill. If possible, paper towels should be used to cover the spill and contain the agent *prior* to leaving the room.
- A solution of 10% household bleach (1:10 dilution) is recommended for cleaning up any spill regardless of the otherwise approved chemical disinfectant.
- *The goal of any spill clean-up is the safety of the researcher and those in the vicinity.* With that in mind, below is the recommended protocol for cleaning up a known or potentially infectious agent.

Any investigator working with microorganisms known to be infectious, or potentially infectious, to humans, animals, or plants should be trained and equipped to deal with spills.

Examples of infectious/potentially infectious materials include:

- Microbiological cultures derived from clinical specimens or pathogenic microorganisms and laboratory equipment that have come into contact with such cultures.
- Tissues, bulk blood, and body fluids from humans and non-human primates.
- Tissues, bulk blood or body fluids from an animal that is carrying an infectious agent that can be transmitted to humans.
- Contaminated sharps.

In any emergency situation, attention to immediate personal danger overrides containment considerations. Currently, there is no known biohazard on the Caltech campus that would prohibit properly garbed and masked fire or security personnel from entering any biological laboratory in an emergency.

Well-prepared staff can appropriately manage the majority of spills. One exception to this general rule is a spill of a significant volume outside of a biological safety cabinet (significance varies depending on the nature of the biohazard, but for purposes of this discussion, we define this to include cultures in excess of three liters in volume).

For spills of this nature:

1. Evacuate the area and post lab with "**DO NOT ENTER**" sign.
2. Call Caltech Security x5000 or the Safety Office x6727.

SMALL BIOHAZARDOUS SPILL OUTSIDE A BIOLOGICAL SAFETY CABINET (BSC)

1. Immediately stop all work and notify co-workers in the immediate area about the spill. If possible, place paper towels on the spill to contain it prior to leaving the area.
2. If necessary, remove contaminated clothing and place into a biohazard bag, wash all contaminated body parts, and flush exposed mucous membranes with water or physiological saline solution.
3. Put on gloves and appropriate personal protective equipment (PPE): protective eyewear, lab coat, mask or face shield (splashing is likely) before starting the spill clean-up.
4. Remove any broken glass or sharp objects from the spill using mechanical means – forceps, hemostats, needle-nose pliers, broom and dust pan. NEVER REMOVE SHARPS/BROKEN GLASS BY HAND!
5. Contain the spill by covering with paper towels and carefully pour appropriate disinfectant solution* around and on the spill area. Take care not to splash disinfectant solution or create aerosols while pouring. Allow proper contact time.
6. Remove the paper towels and repeat the process until all visible contamination is removed. Re-wet cleaned area with disinfectant and air dry or let stand for 10 minutes before wiping dry.
7. Place all contaminated paper towels into a biohazard ("red") bag for appropriate disposal (EHS pick-up and off-site disposal).
8. Remove all PPE into a biohazard ("red") bag for appropriate disposal (EHS pick-up and off-site disposal) and immediately wash hands.

*For most spills, the best disinfectant is a 1:10 solution of household bleach, made fresh. Please consult the Safety Office if you have questions about the best disinfectant for your agent by calling x6727.

LARGE BIOHAZARDOUS SPILL (ONE TO THREE LITERS IN VOLUME) OUTSIDE A BIOLOGICAL SAFETY CABINET (BSC)

1. **Alert co-workers, cover spill with paper towels (to prevent spill from migrating) and leave the lab area immediately;**
2. If applicable, close lab door and post lab with "**DO NOT ENTER**" sign;
3. If necessary, remove contaminated clothing and place into a biohazard bag, wash all contaminated body parts, and flush exposed mucous membranes with water or physiological saline solution;
4. Notify supervisor. If necessary, contact the Safety Office (x 6727) for additional guidance or assistance;
5. **Wait at least 20 minutes prior to re-entry (to allow aerosols to dissipate);**
6. Upon re-entry, don appropriate personal protective equipment (PPE), i.e. lab coat, gloves, and mucous membrane protection (safety glasses and/or face mask, gloves);
7. Carefully pour an appropriate disinfectant solution* onto the towel-soaked spill; care should be taken to minimize splashing. **LET STAND FOR AT LEAST 10 MINUTES;**

8. If broken glass or sharp objects are present, handle with tongs, forceps, brush and dustpan, or other mechanical means. Do not use your hands! Place broken glass in sharps container.
9. Wipe up spill/excess disinfectant working from the outside of the spill toward the center and place paper towels and other contaminated waste into biohazard bag. Spray contaminated surface again with disinfectant and wipe down. Finally, spray area with 70% alcohol and wipe up to remove residual disinfectant;
10. Transfer all contaminated waste into a red biohazard waste container;
11. Wash and mop the entire area around the spill using an appropriate disinfectant;
12. Remove and discard PPE into a red biohazard waste container. Call EHS (x6727) if extra waste pick-up is needed; and
13. Shower or wash hands with soap and water.

*For most spills, the best disinfectant is a 1:10 solution of household bleach, made fresh. Please consult the Safety Office if you have questions about the best disinfectant for your agent by calling x6727.

SPILL IN SMALL LABORATORY EQUIPMENT

Liquid spills on small laboratory equipment shall be contained as follows:

1. Don appropriate PPE (lab coat, gloves, mucous membrane protection);
2. Absorb excess liquid with paper towels;
3. Immerse the contaminated equipment in a 10% bleach solution (made fresh weekly) and allow 10 minutes contact time;
4. Remove equipment from the decontaminant, blot off excess liquid with paper towels;
5. Spray with a 70% alcohol solution, wipe clean to remove potentially corrosive bleach residue;
6. Dispose of paper towels and gloves as biohazard waste; and
7. Wash hands with soap and water.

SPILL IN LARGE LABORATORY EQUIPMENT

Liquid spills on large laboratory equipment (e.g., centrifuge, incubator, autoclave) shall be contained as follows:

1. Don appropriate PPE (lab coat, gloves, mucous membrane protection);
2. Absorb excess liquid with paper towels;
3. Spray the contaminated equipment in a 10% bleach solution (made fresh weekly) including area surrounding the spill;
4. Allow to 10 minutes contact time;
5. Wipe with paper towels;
6. Spray with a 70% ethanol/isopropyl alcohol solution, wipe clean;
7. Dispose of paper towels and gloves as biohazard waste; and
8. Wash hands with soap and water.

B. EXPOSURE RESPONSE PROTOCOLS

Caltech has an Emergency Response Guide flipchart that is posted in each laboratory. The Guide contains procedures for spills, exposure incidents, reporting instructions, and contact numbers. The PI or Lab Safety Officer must show new personnel the location of the Guide and emergency equipment in the laboratory.

Caltech also has an Injury and Illness Prevention Program and the EHS office investigates and follows up on incidents, accidents, and near misses in conjunction with all necessary reporting and follow-up requirements. Please see the Injury and Illness Prevention Program for more information.

Determine the necessity and extent of medical treatment for persons exposed to infectious microorganisms. If it is a life-threatening emergency, call x5000 immediately.

Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation of an infectious agent should be given appropriate first aid.

FIRST AID PROCEDURES

For a Needlestick or a Cut with a Contaminated Sharp

- Immediately wash the area with soap and water;
- Wash the area with appropriate disinfectant (alcohol wipes, iodine pads) for at least 15 minutes;
- Obtain medical attention immediately after washing the area; and
- Report the incident to both your PI and EHS.

For a Splash in the Eye

- Immediately flush the eye with temperate water from the eyewash-station for 15 minutes. If an eyewash-station is not available, use temperate water from the faucet or an emergency eye saline solution for 15 minutes;
- Hold the eyelid open to ensure effective rinsing; and
- Obtain medical attention immediately after rinsing the eye.
- Report the incident to both your PI and EHS.

For Contamination on the Body

- Remove contaminated clothing, shoes, jewelry, etc.;
- Immediately flood exposed skin with water and wash with soap and water for 15 minutes. If a safety shower is not available use the faucet;
- Obtain medical attention immediately after washing; and
- Report the incident to both your PI and EHS

REPORTING INSTRUCTIONS

Report all injuries, accidents, near misses, and exposures to your supervisor and complete the Caltech Accident Report Form. In addition, ensure that all exposure incidents involving recombinant or synthetic organisms and infectious substances are reported to Caltech EHS and the Institute Biosafety Officer as soon as possible for follow up and notification of the appropriate regulatory agency, as necessary.

Any individual receiving an exposure or potential exposure to biohazardous material will be given a medical consultation and advised of available treatment by a physician.

Laboratories that use HIV or HIV derived-virus containing greater than ½ the HIV viral genome must post the HIV Exposure Response Procedure in the laboratory. Personnel exposed or potentially exposed to HIV or HIV pseudovirus must follow the posted procedures.

OBTAINING MEDICAL ATTENTION

1. Call Security at x5000 or 626-395-5000 and indicate the nature of the incident.

2. Security will call 911 if paramedics are necessary, and escort them to you.

3. If you are not able to drive yourself to the clinic, Security can arrange for a taxi and provide you with a voucher for payment

OCCUPATIONAL HEALTH CLINICS

During work hours report to one of the following Pasadena Occupational Health Clinics contracted by Caltech:

- **Pasadena Urgent Care Center** 7:00 AM – 10:00 PM 3160 E. Del Mar Blvd. phone: 626-271-2400

- **St. George's Medical Clinic** 8:30 AM – 6:00 PM 1750 E. Colorado Blvd. phone: 626-440-0097

After 10 PM and before 7 AM, report to **Huntington Memorial Hospital Emergency Room** located at 711 S. Fairmount Ave, Pasadena

CHAPTER VIII: SHIPPING BIOLOGICAL MATERIALS

A. DEPARTMENT OF TRANSPORTATION (DOT) AND INTERNATIONAL AIR TRANSPORT ASSOCIATION (IATA) REGULATIONS

Packaging and shipping regulated biological materials and dry ice must comply with the US Department of Transportation (US DoT) and International Air Transport Association (IATA) regulations. Individuals packaging and shipping regulated biological materials for air transport by a commercial carrier must be trained every two years. Please contact the Safety Office to receive training or more information (safety.training@caltech.edu or x6727).

According to the regulations, packages must be specifically marked and labeled, and packaging materials must be tested for durability and to withstand certain pressures.

The following materials are NOT regulated by DOT or IATA:

- Substances unlikely to contain pathogens, e.g. purified protein or antibodies, or cultures of RG1 microorganisms, such as, *B. subtilis or S. cerevisiae;*
- Neutralized or inactivated substances – Contact the Safety Office to discuss proper means of inactivation;
- Environmental samples (food, water, soil, or dust);
- Blood for transfusion; tissue/organs for transplantation;
- Dried blood spots on filter paper; and
- Biological products subject to FDA approval, e.g. vaccines.

Please note that shipping formalin or ethanol-fixed biological material requires training in shipping those chemicals. Please contact the Safety Office for this training (safety.training@caltech.edu or x6727).

The following materials ARE regulated by DOT and IATA:

- **Category A infectious substance** is an infectious substance capable of causing permanent disability, life-threatening or fatal disease to humans or animals
    - IATA Table 3.6.D provides a list of Category A pathogens; however, this list is not exhaustive and any new or emerging pathogens that meet the definition of the Category must be shipped as such. Examples include a culture of HIV and a culture of Hepatitis B virus (a culture is defined as the result of a process by which pathogens are intentionally propagated.)
- **Category B infectious substance** is an infectious substance which does not meet the criteria for Category A, but is known or suspected of containing pathogens.
    - Patient and animal specimens containing many category A pathogens as specified in IATA Table 3.6.D may be shipped as a Category B biological substance. Examples include blood samples from individuals infected with HIV or Hepatitis B virus.
    - Examples of Category B biological substances include cultures of *S. typhimurium, L.monocytogenes,* and some viral vectors.
- **Exempt human or animal specimens** are patient or animal specimens for which there is minimal likelihood that pathogens are present. Professional judgment is necessary: "Judgment should be based on … the known medical history, symptoms, and individual circumstances of the source, human or animal, and endemic local conditions." - DoT
    - If these specimens are triple packaged with the outer box having at least one surface that is 100 mm, and "**Exempt Human Specimen**" or "**Exempt Animal Specimen**" is written on the outer box, then the remainder of the IATA regulations don't apply
- **Regulated GMMOs, genetically modified micro-organisms that are non-toxic and non-infectious**
    - Organisms/micro-organisms in which genetic material has been purposely altered by a researcher in a way that does not occur naturally. Includes plants, fungi, bacteria, parasites, and animals (flies, worms).
    - If the GMMO is infectious or toxic, it must be sent as either a Category A or B substance.
    - Examples of a GMMO that may be shipped as a GMMO include recombinant *E. coli* K12 or *B. subtilis.*
    - Examples of a GMMO that must be shipped as a Category B biological substance is recombinant *L. monocytogenes.*

B. PACKAGING

Regulated biologicals must be **triple packaged** as follows:

- The primary container is the leak-proof tube that contains the sample. The primary tube or vial must be individually wrapped if it is fragile, e.g. glass, and properly sealed (parafilm);
- Enough absorbent material to absorb all of the liquid in the samples must be placed in the secondary container;
- The secondary container is a leak-proof plastic bag or container;
- The tertiary container is a rigid outer box of good quality with no dents or defects; and
- Depending on the biological substance you are sending, various packaging tests may be required. Contact the Safety Office for training (safety.training@caltech.edu or x6727).

C. ON CAMPUS TRANSPORT BETWEEN BUILDINGS

When transporting biohazardous substances between buildings, the samples must be packaged as outlined below:

- The sample must be in a tightly closed primary container;
- The primary container must be placed in a closed plastic bag or closed plastic container;
- Enough paper towels or other absorbent material to absorb all of the liquid in the samples must be placed in the plastic bag or plastic container;
- If ice or dry ice is needed, place the secondary container within the container of ice;
- Never place dry ice in an air-tight container as carbon dioxide can build-up and the container may over-pressurize;
- The primary, secondary, or outer package must have the agent name and a biohazard label; and
- At least one hand should be un-gloved to open doors during transportation.

D. IMPORTING BIOHAZARD MATERIAL IN THE US

CDC IMPORT PERMIT FOR INFECTIOUS AGENTS

The Centers for Disease Control and Prevention (CDC) Import Permit Program (IPP) regulates the importation of infectious biological agents, infectious substances, and vectors under USPHS 42 CFR - Part 71 final rule February 2013. The IPP may inspect the facility before issuing a permit. Please note that obtaining previously imported material from a colleague in the US may also require a permit. Applications may take at least two weeks to process. The importation permit is necessary for release by US Customs. Please contact the Institute Biosafety Officer with questions or assistance with the permitting process.

Material Requiring CDC Import Permits:

- Any infectious biological agent known or suspected to cause disease in humans.
- Any material known or reasonably expected to contain an infectious biological agent.
- Non-human primate material – all non-human primate material (e.g. blood, plasma, tissue, urine, feces) require an import permit, unless it has been specifically treated and rendered non-infectious.
- Vector - Any animals (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal products (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are known to transfer or are capable of transferring an infectious biological agent to a human.
- Animals – Any member of the animal kingdom except a human including an animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws).
- Arthropods – Any living insect including crustaceans, spiders, scorpions, etc. capable of being a host or vector of human disease.
- Snails – Any freshwater snails (phylum Mollusca, class Gastropoda) capable of transmitting schistosomiasis.
- Bats – All live bats. See below for further information on obtaining an import permit for live bats. Bats may also require a permit from the U.S. Department of Interior, Fish and Wildlife Service. For additional information, see http://www.fws.gov/permits/importexport/importexport.shtml.
- Nucleic acids that can produce infectious forms of any infectious biological agent would require a CDC import permit. For example, viral genomes which consist of positive sense RNA are infectious when the purified viral RNA is applied to permissive cells in the absence of any viral proteins. In some cases, viral genomes which are composed of double-stranded DNA are also infectious (e.g., genome of Cercopithecine Herpesvirus 1 (Herpes B virus).

- **Please note that the described material may require a permit from the United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS) or be prohibited from importation under the USDA regulations. Information on USDA transport or import permits is available at: http://www.aphis.usda.gov/import_export/index.shtml**

Material NOT Requiring CDC Import Permits:

- Diagnostic specimen not known by the importer to contain, or suspected by the importer of containing, an infectious biological agent and is accompanied by an importer certification statement confirming that the material is not known to contain (or suspected of containing) an infectious biological agent, or has been rendered noninfectious.
- Animal or animal product being imported for educational, exhibition, or scientific purposes and is accompanied by documentation confirming that the animal or animal product is not known to contain (or suspected of containing) an infectious biological agent or has been rendered noninfectious.
- Nucleic acids that cannot produce infectious forms of any infectious biological agent and the specimen is accompanied by an importer certification statement confirming that the material is not known to contain (or suspected of containing) an infectious biological agent.
- Animal or animal product listed in 42 CFR Part 71 and its importation has been authorized in accordance with 42 CFR §§ 71.52, 71.53, or 71.56.
- Product that is cleared, approved, licensed, or otherwise authorized under any of the following laws:
  - The Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), or
  - Section 351 of the Public Health Service Act pertaining to biological products (42 U.S.C.262), or
  - The Virus-Serum-Toxin Act (21 U.S.C. 151-159).

CDC Import Permit Application:

- Importation permits are issued only to the importer, who must be located in the United States.
- Phone: (404) 718-2077**;** Fax: (404) 718-2093
- Email: importpermit@cdc.gov
- Website: www.cdc.gov/od/eaipp

OTHER IMPORT PERMITS

The US Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS) permits are required for the import, **domestic transit (transport within the US),** and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms.

Please note that even if your imported biological material does not require a permit, US Customs will not release the package unless a letter that describes the material and declares that it is not considered to be pathogenic or infectious to livestock or poultry is attached to the outside of the package for review by the USDA at the port of entry. Please contact the Safety Office for help with this process. Information on the APHIS permitting requirements is available at: http://www.aphis.usda.gov/permits/index.shtml

The US Food and Drug Administration (FDA) require a permit or registration before importation food (except most meat and poultry), drugs, biologics, cosmetics, and medical devices into the US. See http://www.fda.gov/ora/import/ for more information.

E. EXPORTING BIOLOGICAL MATERIAL

The export of a wide variety of etiologic agents of human, plant, and animal diseases may require a license from the Department of Commerce. You must complete a Caltech Export Form before exporting any material. Information may be obtained from the Caltech Export Compliance Office website, and the Department of Commerce Bureau of Export Administration https://researchadministration.caltech.edu/export, phone x2641, or email exportcompliance@caltech.edu or from the Department of Commerce Bureau of Export Administration at 202-482-0896.

CHAPTER IX: VIRAL VECTORS BIOSAFETY

Viral vectors have become standard tools for molecular and engineer biologists. For this reason, it is necessary that researchers using these biological agents are aware of their origins and the consequences of their use.

The following contains pertinent information for the most commonly used viral vectors at Caltech:

A. ADENOVIRUS

Virology: Medium-sized (90–100 nm), non-enveloped icosahedral viruses containing double-stranded DNA. There are more than 49 immunologically distinct types (6 subgenera: A–F) that can cause human infections. Adenoviruses are unusually **stable to chemical or physical agents** and adverse pH conditions, allowing **for prolonged survival outside of the body.**

Cultivation: Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors is capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant viruses. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

Clinical Features: Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash-associated illnesses. Symptoms of respiratory illness caused by adenovirus infection range from common cold symptoms to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection that can cause more systemic diseases.

Epidemiology: Although epidemiologic characteristics of the adenoviruses vary by type, all are **transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission**. Some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years. Some adenoviruses (e.g., serotypes 1, 2, 5, and 6) have been shown to be endemic in parts of the world where they have been studied, and infection is usually acquired during childhood. Other types cause sporadic infection and occasional outbreaks; for example, epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Epidemics of febrile disease with conjunctivitis are associated with waterborne transmission of some adenovirus types. Acute Respiratory Distress Syndrome (ARDS) is most often associated with adenovirus types 4 and 7 in the United States. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower respiratory tract disease, whereas oral transmission of the virus typically causes no or mild disease.

Treatment: Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

Laboratory Hazards: Ingestion; droplet exposure of the mucous membrane.

Susceptibility to Disinfectants: Susceptible to Accel, 1:10 dilution of household bleach (made fresh), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate. – **NOT susceptible to 70% Ethanol.**

B. ADENO-ASSOCIATED VIRUS (AAV)

Virology: Adeno-associated virus is often found in cells that are simultaneously infected with adenovirus. Parvoviridae; icosahedral, 20–25 nm in diameter; single-stranded DNA genome with protein capsid. AAV

is dependent for replication on the presence of wild type adenovirus or herpesvirus; **in the absence of helper virus, AAV can stably integrate into the host cell genome**. Co-infection with helper virus triggers lytic cycle, as do some agents that appropriately perturb host cells. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theoretical **risk of insertional mutagenesis**.

Clinical Features: No known pathology for wild type AAV serotype 2.

Epidemiology: Not documented. Infection apparently via mouth, esophageal, or intestinal mucosa.

Treatment: No specific treatment.

Laboratory Hazards: Ingestion, droplet exposure of the mucous membrane, direct injection.

Susceptibility to Disinfectants: Susceptible to Accel, 1:10 dilution of household bleach (made fresh), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate. – **NOT susceptible to 70% Ethanol.**

C. LENTIVIRUS

Virology: The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. Five serogroups are recognized, reflecting the mammalian hosts with which they are associated. HIV-1 is the human type species.

Available Constructs: Most of the lentiviral vectors presently in use are HIV-derived vectors. The *cis*- and *trans*-acting factors of lentiviruses are often on separate plasmid vectors, with packaging being provided *in trans*. The vector constructs contain the viral *cis* elements, packaging sequences, the Rev Response Element (RRE), and a transgene. The 2nd generation packaging system combine all the important packaging components: *gag, pol, rev,* and *tat* in one single plasmid. **The 3rd generation packaging system eliminated the Tat protein and expresses *rev* on an independent plasmid. Even though it is more cumbersome to use, this design provides maximum biosafety by further reducing the probability of generating replication-competent virus.**

Lentiviral Pseudotyping: Replacement of the HIV envelope glycoprotein with VSV-G provides a broad host-range for the vector and allows the viral particles to be concentrated by centrifugation.

Clinical Features: In terms of the pathogenesis of lentivirus, some key properties are:

- **Lifelong persistence.** This is a function both of their **ability to integrate into the host chromosome** and evade host immunity. This ability to evade host immunity may be related both to the high mutation rates of these viruses, and to their ability to infect immune cells (macrophages, and in the case of HIV, T-cells).
- **Lentiviruses have high mutation rates**. Lentiviruses replicate, mutate, and undergo selection by host immune responses.
- **Infection proceeds through at least three stages**.
    - Initial (acute) lentivirus infection is associated with rapid viral replication and dissemination, which is often accompanied by a transient period of disease.
    - This is followed by a latent period, during which the virus is brought under immune control and no disease occurs.
    - High levels of viral replication then resume at some later time, resulting in disease.

Epidemiology: Transmitted from person to person through direct exposure to infected body fluids (blood, semen), sexual contact, sharing unclean needles, etc.; transplacental transfer can occur.

Laboratory Hazards: Direct contact with skin and mucous membranes of the eye, nose, and mouth; accidental parenteral injection; ingestion; hazard of aerosols exposure is unknown.

Susceptibility to Disinfectants: Susceptible to many disinfectants – Accel, 1:10 dilution of household bleach (made fresh), 70% ethanol, 2% glutaraldehyde/formaldehyde.

Please note that if the lentivirus is carrying an oncogene or potential oncogene, an exposure could result in the oncogene integrating into your genome. A lentivirus harboring an oncogenic transgene is likely one of the most hazardous viral vector constructs used at Caltech.

Use of lentivirus at Caltech must be approved by the IBC prior to initiation of the work and requires laboratories operating at Biosafety Level 2 with Biosafety Level 3 practices.
Please contact the Biosafety Officer for more information.

D. RETROVIRUS (OTHER THAN LENTIVIRUS)

Retroviruses are infectious viruses that integrate into transduced cells with high frequency and may have oncogenic potential in their natural hosts. Retrovirus vectors are usually based on murine viruses. They include ecotropic viruses (infect murine cells only), amphotropic viruses (infect murine and human cells), or pseudotyped viruses, when vector particles express glycoproteins derived from other enveloped viruses (usually can infect human cells). The most common glycoprotein currently used is VSV-G; however, there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola, and Marburg.

Virology [Moloney Murine Leukemia Virus (MoMuLV), Murine Stem Cell Virus (MSCV), etc.]: Retroviridae; subfamily oncoviridae type C, enveloped, icosahedral core, virions 100 nm in diameter, diploid, single-stranded, linear RNA genome. MoMuLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

Virus is not lytic. Data suggest a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk.

The host range is dependent upon the specificity of the viral envelope. The ecotropic *env* gene produces particles that infect only rodent cells. The amphotropic *env* gene allows infection of rodent and non-rodent cells, including human cells.

VSV-G envelope allows infection in a wide range of mammalian (including human) and non-mammalian cells.

Clinical Features: None to date.

Epidemiology: MoMuLV infects only actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germ-line infection. *In vivo* transduction in humans appears to require direct injection with amphotropic or pseudotyped virus.

Treatment: No recommended treatment.

Laboratory Hazards: Contact with feces or urine from infected animals for 72 hours post-infection. Contact with tissues and body fluids of infected animals. Direct injection.

Susceptibility to Disinfectants: Susceptible to many disinfectants – Accel, 1:10 dilution of household bleach (made fresh), 70% ethanol, 2% glutaraldehyde/formaldehyde.

For more details on biosafety features for other viral vectors, please contact the Safety Office (x6727)

CHAPTER X: BIOLOGICAL TOXINS

A. BASIC CHARACTERISTICS

Biological toxins are natural, poisonous substances produced as by-products of microorganisms (exotoxins, endotoxins, and mycotoxins, such as T-2 and aflatoxins), plants (plant toxins such as ricin and abrin), and animals (zootoxins such as marine toxins and snake venom). Unlike pathogenic microorganisms, including those that produce toxins, the **toxins themselves are not contagious and do not replicate**. In this regard, toxins behave more like chemicals than infectious agents. However, unlike many chemical agents, biological toxins are not volatile and are odorless and tasteless. The stability of toxins varies greatly, depending on the toxin structure (low molecular weight toxins are quite stable).

Most biological toxins, with the exception of T-2 Mycotoxin, are NOT dermally active; i.e., intact skin is an excellent barrier against most toxins. That said, mucous membranes of the eyes, nose, and mouth serve as portals of entry, as do breaks in the skin. Aerosol transmission, ingestion, and percutaneous transmission are also a concern for most biological toxins.

Bacterial toxins can be exotoxins (including enterotoxins) or endotoxins. Exotoxins are cellular products excreted from certain viable Gram-positive and -negative bacteria, highly toxic (i.e., microgram quantities) and are relatively unstable (destroyed rapidly when heated to ≥ 60^{o}C). Bacterial endotoxins are lipopolysaccharide complexes derived from the cell membrane of Gram-negative bacteria that are released upon bacterial death. Endotoxins are relatively stable (can withstand heating at 60^{o}C for hours without losing activity) and moderately toxic (tens to hundreds of micrograms required for animal fatality).

The modes of action of biological toxins vary, but include damage to cell membranes or cell matrices (e.g., *Staphylococcus aureus* alpha toxin), inhibition of protein synthesis (e.g. Shiga toxin), or via activation of secondary messenger pathways (e.g. *Clostridium botulinum* and *C. difficile* toxins).

B. LABORATORY REQUIREMENTS AND SAFETY OPERATIONS

Most work with biological toxins can be safely managed in a BSL2 setting. In some cases (e.g., large scale production, manipulation of large quantities of powder form of toxin) management at BSL3 may be required, depending on the toxin in question and the quantities used.

The most hazardous form of any toxin is the dry, powder form. Manipulations of dry forms of toxins should be performed in a biological safety cabinet or in a fume hood. In some cases a glove box may be recommended for such operations.

Once reconstituted into an aqueous form, BSL2 management is usually sufficient for work with most biological toxins. Access to the lab should be controlled when toxin is in use. Biohazard warning signs displaying the biosafety level, toxin in use, emergency contact information, and entrance requirements should be posted at the lab entrance. If vacuum lines are used, protect the vacuum system with an in-line disposable HEPA filter.

Personal protective equipment should include a lab coat, gloves, and mucous membrane protection.

All personnel in the lab should be trained about the specific hazards associated with the toxin in use.

At Caltech, an IBC protocol is required for research utilizing any of the toxins listed in the following table.

TOXINS THAT REQUIRE AN IBC PROTOCOL

Toxin	LD_{50} (µg/kg)
Abrin	**0.7**
Aerolysin	7
Botulinum toxin A	**0.0012**
Botulinum toxin B	**0.0012**
Botulinum toxin C1	**0.0011**
Botulinum toxin C2	**0.0012**
Botulinum toxin D	**0.0004**
Botulinum toxin E	**0.0011**
Botulinum toxin F	**0.0025**
b-bungarotoxin	14
Clostridium difficile enterotoxin A	0.5
Clostridium perfringens lecithinase	3
Clostridium perfringens perfringolysin O	13-16
Clostridium perfringens delta toxin	5
Clostridium perfringens epsilon toxin	0.1
Conotoxin (Only short, paralytic alpha conotoxins with specific sequences are considered Select Agents)	**12-30**
Diacetoxyscirpenol	**1000-10,000**
Diphtheria toxin	0.1
Listeriolysin	3-12
Modeccin	1-10
Pertussis toxin	15
Pneumolysin	1.5
Pseudomonas aeruginosa toxin A	3
Ricin	**2.7**
Saxitoxin	**8**
Shiga toxin	0.25
Shigella dysenteriae neurotoxin	1.3
***Staphylococcus* enterotoxin B**	**25**
***Staphylococcus* enterotoxin F**	**2-10**
***Staphylococcus* enterotoxins A, C, D, and E**	**20(A); <50(C)**
Streptolysin O	8
Streptolysin S	25
T-2 toxin	**5,000-10,000**
Taipoxin	2
Tetanus toxin	0.001
Tetrodotoxin	**8**
Volkensin	1.4
Yersinia pestis murine toxin	10

Toxins noted in RED are considered Select Agents if being stored in large enough quantities (see Chapter XI.B below). For more information please consult: http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html

C. SECURITY

It is important that stocks of biological toxins be maintained in locked cabinets, freezers, and/or refrigerators. Since biological toxins are not self-replicating as are microorganisms, **it is prudent to maintain an inventory or "record of quantity used" to allow the proper assessment of the quantity of toxin present in a lab at any given time**. This inventory should display the current quantity of a particular toxin on-site, the date and amount removed from storage, the person removing the aliquot from storage, the purpose of use, and the quantity remaining. *Toxin Inventory* forms are available from the Safety Office upon request.

D. DECONTAMINATION METHODS

The majority of biological toxins can be inactivated or decontaminated with household bleach. Tables below describe the inactivation regimens for biological toxins in common use:

COMPLETE INACTIVATION OF DIFFERENT TOXINS WITH A 30-MINUTE EXPOSURE TIME TO VARYING CONCENTRATIONS OF SODIUM HYPOCHLORITE (NaOCl) +/- SODIUM HYDROXIDE (NaOH)

Toxin	2.5% NaOCl[a] + 0.25 N NaOH	2.5% NaOCl[a]	1.0% NaOCl[b]	0.1% NaOCl[c]
T-2 Mycotoxin	YES	NO	NO	NO
Brevetoxin	YES	YES	NO	NO
Microcystin	YES	YES	YES	NO
Tetrodotoxin	YES	YES	YES	NO
Saxitoxin	YES	YES	YES	YES
Palytoxin	YES	YES	YES	YES
Ricin	YES	YES	YES	YES
Botulinum	YES	YES	YES	YES

(Wannemacher 1989)

[a]2.5% NaOCl is approximately equal to 50% household bleach (1:2 dilution)

[b]1.0% NaOCl is approximately equal to 20% household bleach (1:5 dilution)

[c]0.1% NaOCl is approximately equal to 2% household bleach (1:50 dilution)

For exposure events involving skin exposure to minute quantities of toxin, soap and water are effective in removing the toxin burden (toxins are not dermally active, except for T-2 mycotoxin). For significant exposures to biological toxins, contact both your PI and the Safety Office, and seek medical attention immediately.

CHAPTER XI: RESEARCH COMPLIANCE

All research activities undertaken by faculty, staff, and students at Caltech should be conducted in accordance with strict ethical principles and in compliance with federal and state regulations and Institute policies. The Office of Research Compliance, which reports to the Vice Provost for Research, is responsible for providing support and training to faculty, students and staff in order to meet these requirements and maintain a robust research compliance program at Caltech.

The Office of Research Compliance works with faculty oversight Committees to promote the ethical and responsible conduct of research and to ensure compliance with regulatory requirements relating to research involving human and vertebrate animal subjects, recombinant DNA, biohazards, radioactive materials, and stem cells. The Committees supported by this office include the Institutional Animal Care and Use Committee (IACUC), the Institutional Review Board (IRB), and the Administrative Committee on Biosafety (IBC and IRE). The Office of Research Compliance also has responsibilities relating to responsible conduct of research, conflicts of interest, controlled substances, compliance with U.S. export control regulations, and third party use of Caltech's research facilities.

A. NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH AND THE INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

The NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules contain procedures for the safe construction and handling of recombinant and synthetic nucleic acid molecules and for the cells, organisms, and viruses that contain them. The Guidelines apply to Caltech and all institutions that receive NIH funding for recombinant or synthetic nucleic acid molecules research. Consequences of noncompliance include suspension, limitation, or termination of NIH funds for recombinant and synthetic nucleic acid research at the Institution, or a requirement for prior NIH approval of recombinant or synthetic nucleic acid research at the Institution.

The purpose of the Guidelines is to specify safe handling practices and containment levels for recombinant or synthetic nucleic acid molecules, organisms and viruses containing recombinant and synthetic nucleic acid molecules, and transgenic animals, vertebrates and invertebrates, that are covered under the Guidelines. The NIH Guidelines mandate research institutions to form and administer an Institutional Biosafety Committee (IBC) to oversee the use and safety measures associated with the use of rDNA in research activities, at the Institutional level. Research involving recombinant or synthetic nucleic acids is covered under one of six sections (Sections III-A through III-F) of the NIH Guidelines for the Use of Recombinant DNA Molecules. Research oversight can be extended to other research hazards at the discretion of the Committee.

The Principal Investigator (PI) is responsible for submitting an IBC Protocol Submission to the IBC, highlighting the use of recombinant DNA and other Biohazardous materials.

The IBC reviews, approves, and oversees the proposed research to ensure compliance with the Guidelines, determine necessity of health surveillance of personnel, ensure training for IBC members, staff, PIs, and laboratory staff, and set biosafety containment levels as required by the Guidelines.

The Institute Biosafety Officer conducts lab inspections, develops emergency and reporting procedures, investigates lab accidents, reports recombinant and synthetic nucleic acid incidents, violations of the Guidelines to the IBC and provides biosafety training to IBC members, PIs, and laboratory staff. This is a summarized description of the IBC and Biosafety review process. For more details please see http://ibc.caltech.edu/

INCIDENT REPORTING TO NIH

The following incidents require **immediate reporting** to National Institute of Health Office of Science Policy NIH OSP:

- Spills or accidents involving recombinant and synthetic nucleic acids covered under the Guidelines that require BSL2 containment and resulted in an overt exposure, e.g., needlestick; splash in eyes, nose, mouth; or accidental aerosolization/inhalation.
- Spills or accidents involving recombinant and synthetic nucleic acids requiring BL3 or BL4 containment resulting in an overt exposure or potential exposure, e.g., spills of high risk recombinant materials occurring outside of a biosafety cabinet.

The following incidents must be reported to NIH OBA **within 30 days**:

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

Minor spills of low-risk agents, contained and properly disinfected, generally don't need to be reported to the NIH. NIH OBA is available for consultation if there is uncertainty whether an incident is reportable. The incident report to NIH OBA can be submitted by the Institution, IBC, BSO, or PI. The report should include the response made to mitigate the problem and preclude its reoccurrence.

For more information contact the Biosafety Officer or the IBC Administrator or see http://ibc.caltech.edu/

B. SELECT AGENT REGULATION

The Federal Government has published a list of infectious agents and biological toxins that it strictly regulates due to their potential for use as bioterror agents. Shipping, manipulation, and even possession of these "Select Agents" are heavily regulated at the Federal and Institutional level. Currently, there are no viral or bacterial Select Agents that are currently approved for use on the Caltech campus.

For more information about the National Select Agent Program, including a list of the agents that are currently regulated, please visit this site: http://www.selectagents.gov/index.html

The toxins listed below are exempt from CDC and USDA registration Select Agent requirements if the maximum allowable exempt quantity **per Principal Investigator** is not exceeded. **PIs must keep toxin locked and maintain inventories to ensure maximum exempted amount is not exceeded.** Please contact the Institute Biosafety Officer for help in developing SOPs for the select agent toxins. Use of exempt amounts of select agent toxins must be registered with the IBC.

Toxin	**Maximum Exempted Amount per PI**
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Conotoxins (short, paralytic alpha)	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Staphylococcal enterotoxins subtypes A-E	5.0 mg
Tetrodotoxin (TTX)	100 mg
T-2 toxin	1000 mg

If you think you may be in possession of agents on the Select Agent list or a quantity of Select Toxins above the exempted amount, or if you intend to initiate research activity with any of this items, please contact the Safety Office at x6727.

C. DUAL USE RESEARCH OF CONCERN - DURC

Broadly defined, "dual use" refers to the malevolent misapplication of technology or information initially developed for benevolent purposes. In the realm of life sciences, "dual use" refers to the potential misuse

of microorganisms, toxins, recombinant or synthetic nucleic acid technology or research results to threaten public health or national security. “Dual Use Research of Concern,” referred to as DURC, is research that has a potential to be DIRECTLY misapplied.

Caltech Institutional Oversight of Life Sciences Dual Use Research of Concern Policy is based on recommendations and guiding principles from The United States Government (*March 2012 DURC Policy* and *September 2014 Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern)*.

Caltech Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern articulates the practices and procedures required to ensure that Dual Use Research of Concern is identified at the institutional level and risk mitigation measures are implemented as necessary.

The purpose of this Policy is to describe and frame ongoing institutional review and oversight of certain life sciences research with high-consequence pathogens and toxins in order to identify potential DURC and mitigate risks where appropriate. This Policy delineates the roles and responsibilities of Caltech Research Administration, the Principal Investigators (PIs) engaged in research activity that can have DRUC potential or that has been identified as DURC, and the Caltech DURC Committee.

The Policy seeks to preserve the benefits of life sciences DURC while minimizing the risk that the knowledge, information, products, or technologies generated from such research could be used in a manner that results in harm to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security.

Under this Policy, review will focus on research that involves one or more of the following Select Agents or toxins:

Avian influenza virus-highly pathogenic
Bacillus anthracis
Botulinum neurotoxin*
Burkholderia mallei
Burkholderia pseudomallei
Ebola virus
Foot-and-mouth disease virus
Francisella tularensis
Marburg virus
Reconstructed 1918 Influenza virus
Rinderpest virus
Toxin-producing strains of *C. botulinium*
Variola major virus
Variola minor virus
Yersinia pestis

*For the purposes of the DURC Policy, there are no exempt quantities of botulinum neurotoxin. Research involving any quantity of botulinum neurotoxin should be evaluated for DURC potential.

Planned and on-going experiments, as well as data obtained from these experiments, should be evaluated for their potential to:

- Enhance the harmful consequences of the agent or toxin;
- Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification;
- Confer to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
- Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
- Alter the host range or tropism of the agent or toxin;
- Enhance the susceptibility of a host population to the agent or toxin; and
- Generate or reconstitute an eradicated or extinct agent or toxin listed above.

PI Responsibilities:
Assess their own research and the research of those under their supervision for dual use potential and report as appropriate:

- Stay abreast of literature, guidance, and requirements related to dual use research, and particularly Dual Use Research of Concern (DURC);

- Ensure that their lab personnel are able to identify DURC and manage it properly (see https://researchcompliance.caltech.edu/documents/51-durc_policy.pdf);
- Conduct research responsibly, especially research that may meet the criteria for DURC;
- Give thought as to how the results of such research should be communicated to others, including the public; and
- Always be alert to potential misuse of research.

5 Key Questions to Assess DURC Risk:

1. Could this research yield information that could be intentionally misused to threaten public health, safety, and/or security?
2. What is the nature of the threat that could be posed from intentional misapplication of the information and what are the potential consequences?
3. Based on questions 2&3, how reasonably anticipated is it that the information could be used to pose a threat to public health, safety, and/or security?
4. Could this research yield information that could potentially benefit the life sciences and/or public health, safety, or national security?
5. Do the potential risks outweigh the potential benefits?

For help evaluating or registering DURC experiments, contact the Institute Biosafety Office or the Office of Research Compliance.

Biosafety Resources

American Biological Safety Association (ABSA): http://www.absa.org/

ABSA Biosafety Links: http://www.absa.org/resbslinks.html#bmbl

Biosafety in Microbiological and Biomedical Laboratories: http://www.cdc.gov/biosafety/publications/bmbl5/

California Medical Waste Management Act:
http://www.cdph.ca.gov/certlic/medicalwaste/Documents/MedicalWaste/2013/MWMAfinal2013.pdf

California Medical Waste Program: http://www.cdph.ca.gov/certlic/medicalwaste/Pages/default.aspx

National Select Agent Program: http://www.selectagents.gov/Select%20Agents%20and%20Toxins.html

NIH Guidelines: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

NIH Office of Biotechnology Activities (OBA): http://oba.od.nih.gov/oba/index.html

NIH/OBA Frequently Asked Questions: http://oba.od.nih.gov/rdna/rdna_faq_list.html

NIH/OBA IBC Information: http://oba.od.nih.gov/rdna_ibc/ibc.html

NIH/OBA Educational Materials: http://oba.od.nih.gov/rdna_ibc/ibc_training.html

NIH/OBA Dual Research: http://oba.od.nih.gov/biosecurity/biosecurity.html

Primary containment for biohazards: selection, installation, and use of biosafety cabinets:
http://www.cdc.gov/biosafety/publications/index.html

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