

California Institute of Technology

BIOSAFETY MANUAL



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CHAPTER I: INTRODUCTION

The purpose of the California Institute of Technology's (Caltech or Institute) 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 the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (1) and the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL, 6th Edition) (2) to assure 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 that 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 materials, 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, online and hands-on, Institute wide and lab specific);**
- **Completion of all applicable Occupational Health requirements, including documentation of required physicals, medical clearances, and/or vaccinations;**
- **Immediate reporting to the Principal Investigator or supervisor of any situation that compromises an individual's ability to perform as required in a BSL2 or ABSL2 laboratory, including physical or psychological issues; and**
- **Immediate reporting to the Principal Investigator, supervisor 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://wellness.caltech.edu/counseling>).

Another important institutional mechanism is a formal, anonymous reporting system for instances of noncompliance with established Caltech safety and/or security policies in the laboratory. At Caltech, multiple pathways for anonymously reporting behaviors of concern exist, depending on the situation, including:

- (1) Reporting to your PI/Supervisor;
- (2) Reporting to your Department/Division Operation Officer and/or Division Chair;
- (3) Reporting to the Caltech Campus Anonymous Hotline (<http://hotline.caltech.edu>, x8787);
- (4) Reporting to the Institute Biosafety Officer, the Chief Research Policy Officer, or any members of the Institutional Biosafety Committee (IBC); and
- (5) Reporting to the Environmental Health and Safety Office (EH&S).

ROLES & RESPONSIBILITIES

1. Principal Investigator

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

The PI is responsible for

- Complying with all applicable state and federal regulations and guidelines;
- Ensuring the safe operation of their laboratory;
- Assessing the risks of their ongoing or planned experiments and conveying those risks to lab personnel;
- Providing lab-specific orientation to new lab personnel including the location of the Emergency Response Guide and emergency equipment located in the laboratory; and
- Registering and obtaining approval with the IBC for experiments falling within the *NIH Guidelines* (1) or otherwise within the IBC's purview (<https://ibc.caltech.edu>).

2. Laboratory Safety Coordinator

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

- Promulgating safety information to other laboratory members;
- Providing lab-specific onboarding to new lab members, including the location of Emergency Response Guide and emergency equipment located in the laboratory;
- Acting as a liaison with EH&S personnel, the Institute Biosafety Officer, the IBC, or the Office of Research Compliance on behalf of the PI;
- Acting as the lab emergency coordinator and liaison with the Caltech Emergency Response Network; and
- Facilitating periodic, ongoing biosafety laboratory inspections.

For more information about Laboratory Safety Coordinators, click [here](#).

3. Laboratory Personnel

All laboratory personnel, including students, who work with biohazardous materials in research

laboratories are responsible for the following:

- Complying with safety rules, regulations, and procedures required for the task(s) assigned;
- Knowing and understanding the hazards of materials and processes prior to conducting work and utilizing appropriate measures to control these hazards;
- Attending required [Safety Training](#);
- Using appropriate personal protective equipment (PPE);
- Participating in medical surveillance when required;
- Reporting accidents, injuries, or near misses to the PI and the EH&S Office;
- Reporting unsafe conditions to the PI or immediate Supervisor and the EH&S Office; and
- Keeping work areas safe and uncluttered and maintaining appropriate hygiene practices.

4. Division Operations Officers

It is the responsibility of the Division Operations Officers to ensure their Divisions conduct operations in accordance with applicable laws and regulations and to implement the Institute's environmental, safety, and emergency policies. Additionally, Division Operations Officers 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 with Caltech's policies, procedures, programs, and practices;
- Ensuring that workplace safety and health practices and procedures are clearly communicated and understood by employees through ongoing 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 within 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 maintained; and
- Ensuring that inspections/investigations and employee records are kept for the designated time period(s) per the Institute Record Retention Guidelines.

5. Institute Biosafety Officer

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

- Providing technical guidance on matters pertaining to biosafety and biosecurity;
- Preparing, administering, and overseeing Institutional implementation of the Biosafety Manual;
- Periodically reviewing the Biosafety Manual and revising it as necessary;
- Conducting root cause analysis for accidents, illnesses, or near misses involving biohazardous materials;
- Providing and coordinating biosafety training including Biosafety Principles training, *NIH Guidelines* training, Bloodborne Pathogens training, Aerosol Transmissible Diseases training, Viral Vectors training, and Biological Toxins training;
- Performing observational surveys/inspections of laboratories and proposing corrective actions;
- Assisting PIs with risk assessments;
- Assisting research staff with submission of registrations to the IBC and maintaining registration files;
- Assisting the Office of Research Compliance with reporting recombinant or synthetic nucleic acid incidents and violations of the *NIH Guidelines* to the IBC or, on behalf of the IBC, to the NIH Office of Science Policy;

- Identifying concerns or gaps in compliance; and
- Developing emergency and reporting procedures.
- Delegating, if needed, part of the duties and tasks described above to the Assistant Biosafety Officer (ABSO).

CHAPTER III: GENERAL BIOSAFETY PRINCIPLES

A. RISK ASSESSMENT

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

- The biological and physical hazard characteristics of the agent,
- The sources likely to harbor the agent,
- The susceptibility of the potential incidental host,
- The procedures that may disseminate the agent, and
- 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, and parasites, among 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.

The Risk Group listing (table shown below) of the *NIH Guidelines* is an accepted standard and can be accessed electronically at <https://osp.od.nih.gov/biotechnology/nih-guidelines/>.

Table 1: Risk Group Listing (NIH)

| | |
|------------|--|
| RG1 | Agents are not associated with disease in healthy adult humans. Examples: <i>Bacillus subtilis</i> , <i>E. coli</i> 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: <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> spp. |
| RG3 | Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. Examples: <i>Mycobacterium tuberculosis</i> , HIV, <i>Yersinia pestis</i> , <i>Bacillus anthracis</i> , Avian Influenza H5N1 |
| 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, Herpes B virus |

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 organisms 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 immunity 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. ROUTES OF INFECTION

Infectious disease transmission is directly linked to the route utilized by a pathogen to enter the body of its host. There are five main routes of disease transmission: aerosol, ingestion, direct contact, fomite, and vector-borne. It is critical for prevention and containment practices to account for route of infection for each pathogen to minimize the risk of transmission of the infectious material. **Various pathogens will exhibit greater abilities to transmit upon various exposure routes. Appropriate precautions should be implemented accordingly to reduce the risk of such exposures.**

| | |
|--------------------------|---|
| 1. Aerosol | Infectious agents contained in aerosol droplets are transmitted to incidental hosts upon direct entry to the respiratory tract. |
| 2. Ingestion | Infectious material in contaminated food and drinks, or mouth contact with contaminated hands, leads to the entry of the infectious agents through the gastrointestinal tract. |
| 3. Direct Contact | Hosts become infected after direct physical contact with the infectious material. Some agents can enter upon contact with intact skin, open wounds, or mucous membranes (nose, mouth, or eyes). |
| 4. Fomite | A contaminated inanimate object transmits infectious agents to a susceptible host. It usually involves a secondary route of transmission (direct or ingestion) for the pathogen to enter the host. |
| 5. Vector-borne | An insect, for example, transmits a pathogen, either mechanically or biologically, to a host. Mechanical transmission: disease agent is transported by the vector. Biological transmission: disease agent is taken up by the insect (usually blood meal), replicated, and then regurgitated onto the next host via bites. |

C. EXPOSURE SOURCES

Research laboratories use a large variety of biological and biohazardous material. The nature, origins, format, and scale of the material must be considered for conducting a risk assessment.

1. Human Pathogens

Microorganisms (e.g., fungi, bacteria, virus, parasites, rickettsia, prions) classified at RG2 and above can

cause infection and disease in humans.

2. Plant and Animal Pathogens

Plant and animal pathogens may be classified as RG1 (or above) and could be handled at BSL1 or above. A USDA/APHIS permit is required for the use of plant pathogens regardless of the biosafety level. Contact the EH&S Office or the Office of Research Compliance for help in completing the permit application form. **All plant and animal pathogens classified as RG2 must be registered with the IBC prior to handling in the laboratory.**

RG2 organisms are not the only biohazard exposure sources in research laboratories. When performing the laboratory's biohazard risk assessment, also consider the following:

3. 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, saliva, 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 bodily fluids are treated as if known to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (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 Cal/OSHA Bloodborne Pathogens (BBP) Standard (California Code of Regulations, Title 8, Section 5193) applies to all occupational exposure to human blood or other potentially infectious human materials. Under the Cal/OSHA BBP Standard, Caltech is required to develop and maintain a written Exposure Control Plan, offer employees the Hepatitis B vaccination when applicable, and provide annual training and post-exposure medical evaluation. For more information on the Cal/OSHA BBP Standard, see the [Caltech BBP Exposure Control Plan](#).

Animals may harbor endogenous pathogens that are harmful 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.** Work with non-human primate unfixed tissues should be registered with the IBC, and a B virus-specific training may be required prior to handling the samples.

For both human and animal tissues, a risk assessment should be conducted to also identify the potential for aerosol transmissible pathogens (ATP). The Cal/OSHA Aerosol Transmissible Diseases (ATD) Standard (California Code of Regulations, Title 8, Section 5199) might apply if samples are reasonably expected to contain microorganisms that spread via inhalable particles and droplets.

4. Cell 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 from 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.

When a cell culture is inoculated with (or known to contain) an etiologic agent with a higher biosafety

level, it should be classified and handled at the same biosafety level as the agent.

OSHA considers both primary and established human cell lines to potentially contain BBPs. Therefore, laboratory personnel handling cell cultures of this type are required by law (California Code of Regulations, Title 8, Section 5193) to undergo BBP training. At Caltech, this training requirement can be satisfied by attending an in-person training session. For training information go to [EH&S BBP Training](#).

5. 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 tissue processing, 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 or when simply handling the animals.

6. Aerosol Transmissible Diseases

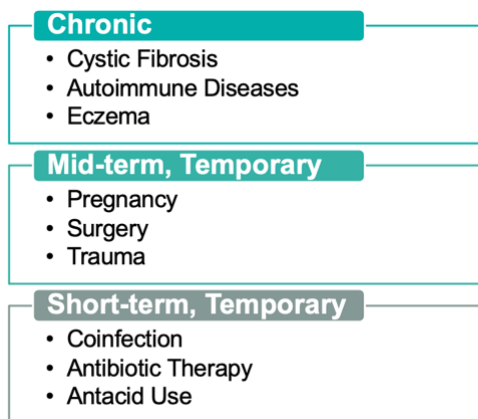
The California Code of Regulations, Title 8, Section 5199 requires that Caltech identifies and minimizes occupational exposure to infectious diseases that spread via inhalable particles and droplets. A specific [Aerosol Transmissible Diseases \(ATD\) Exposure Control Plan](#) is maintained by EH&S and applies to all Caltech research laboratories handling infectious material known to transmit via aerosol (see [Appendix D](#)). This plan is specifically designed to eliminate or reduce occupational exposures to ATDs and sets forth procedures, control measures, and equipment designed to eliminate or minimize risk from exposure to known pathogens such as influenza virus, *Legionella pneumophila*, etc. and to novel pathogens such as SARS-CoV-2, the causative agent for COVID-19.

D. OCCUPATIONAL HEALTH PROGRAM IN SUPPORT OF BIOMEDICAL RESEARCH

According to the BMBL 6th Edition, **a robust and comprehensive occupational health program is integral in the promotion of a workplace culture of safety in biomedical and microbiological research.** An occupational health program that supports staff with access to biological hazards, such as infectious agents or toxins, should aim to alleviate the risk of adverse health consequences due to potential exposures to biohazards in the workplace. Health services should be risk-based and tailored to meet the needs of individual staff and the research institution based on risk assessment. Different elements of occupational health support may be indicated at various stages of employment, ranging from anticipatory risk mitigation (e.g., preplacement evaluation or vaccination) to incident-driven medical measures such as post-exposure immuno- or chemoprophylaxis.

Several aspects are therefore considered for implementing a robust occupational health support.

1. Research/Lab Personnel Health Status



**Consult with
your doctor
or an
occupational
health
provider**

Some unusual circumstances or projects 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 and strongly recommended practice to inform your personal physician or Caltech Occupational Medical Practitioner about your occupational risks, especially work with biohazardous or potentially biohazardous agents, so they have a record of this information.

Certain medical conditions increase risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can be chronic or temporary and 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.

2. Medical Surveillance and Prophylaxis Strategies

Continual collaboration among stakeholders is key to optimal protection of research staff. A designated occupational healthcare provider should work with institutional safety staff, principal investigators (PIs), and other SMEs to ensure optimal work-related health care of laboratorians and their support staff. Caltech's program is designed to implement promptly any indicated pre- and post-exposure medical measures and related counseling. Caltech contracts with commercial occupational health providers to provide occupational health services for personnel who are occupationally at risk of exposure to BBPs, ATDs, and other biohazardous agents. Any individual who experiences an exposure or potential exposure will be provided with medical consultation and advised on available treatments, as applicable.

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, seasonal influenza vaccine, SAD rabies vaccine, MMR vaccine, and HIV or TB testing.

3. Pre- and Post-Exposure Communications

All research laboratories are encouraged to maintain a laboratory-specific biosafety manual that specifies the steps all staff should take immediately after an incident. An effective incident response, including medical care of affected staff, relies on the coordinated execution of the plan and concise, prompt communications. Laying the foundation for proper post-exposure risk mitigation begins before an occupational exposure occurs (e.g., with risk awareness training in the workplace and targeted preplacement occupational health evaluations). Incident response protocols are in place and describe requisite notifications at the time of a potential exposure, including how to access medical care.

4. Laboratory Animal Users Occupational Health Program (LAOHP)

Caltech is required by the Public Health Service Policy to provide an occupational health and safety program specifically designed for all personnel working with laboratory animals. The program protects the health of people that have direct contact with laboratory animals and personnel accessing animal housing area on a regular basis. The LAOHP risk assessment discusses agent-specific risk factors and incidental hazards (e.g., zoonotic infections, toxic chemicals, or laboratory animal allergens), and

dispenses information on health conditions that might increase susceptibility to infection and complications after an occupational exposure. 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 at <http://safety.caltech.edu/root-pages/lab-animal-occupational-health-program>.

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

5. Caltech's contracted Occupational Health Providers

Concentra Urgent Care — 9350 Flair Dr., Unit 102, El Monte 91731 / 626-407-0300

NOTE: There are numerous Concentra locations throughout Los Angeles. [Click](#) for list of Occupational Health Service locations and hours of operation.

Huntington Memorial Hospital – ER — 711 S. Fairmount Ave., Pasadena 91105 / 626-397-5000

CHAPTER IV: BIOHAZARD CONTAINMENT

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

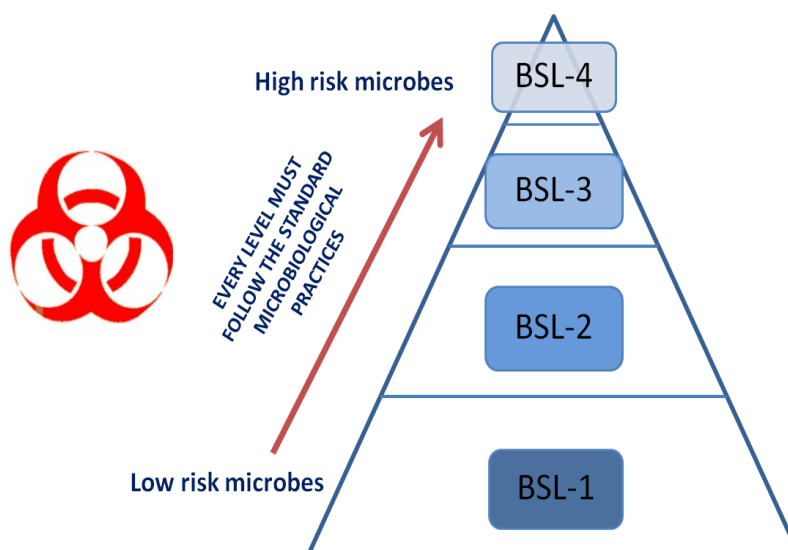
A. BIOSAFETY LEVELS

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 the specific lab activity.

It is the combination of practice, equipment, and facility design that forms 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 to health and safety.



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 an RG3 pathogen may render it safe to be handled with a BSL2 facility and practices. Conversely, insertion of toxin-producing genes in an RG1 microorganism may require a BSL2 facility and practices. Furthermore, RG2 agents with high potential of causing mutagenesis or oncogenesis may require additional BSL3 practices in a standard BSL2 facility.

The Institutional Biosafety Committee (IBC), established under the *NIH Guidelines*, assists Principal Investigators and Laboratory supervisors in determining the proper Biosafety Level for a particular project. One should always carefully review the approved, project-specific IBC protocol prior to starting the research. For information about your IBC protocol, email the IBC administrator at ibc@caltech.edu or contact the BSO.

B. SUMMARY OF BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

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 table below.

| BSL | LABORATORY PRACTICES | PRIMARY BARRIERS AND SAFETY EQUIPMENT | FACILITIES (secondary barriers) |
|-----|---|--|--|
| 1 | <p>Standard Microbiological Practices</p> <ul style="list-style-type: none"> • Proper housekeeping • No food/drink in the lab • Proper hand washing | <p>None Required (open bench work)</p> <p><u>PPE should be considered for chemical hazards</u></p> | <p>Hand washing sink Eyewash station</p> |
| 2 | <p>BSL1 Practices Plus:</p> <ul style="list-style-type: none"> • Access control • Biohazard signage • Universal precautions for handling sharps • Biosafety Manual • Waste management • Medical surveillance | <p>Primary Barriers:</p> <ul style="list-style-type: none"> • Class II biosafety cabinet or other physical containment devices used for manipulation of agents with high potential of splashes or aerosols of infectious materials. • PPE: Laboratory coats & gloves; face protection as needed | <p>BSL1 Plus:</p> <ul style="list-style-type: none"> • Required negative air flow into lab. |
| 3 | <p>BSL2 Practices Plus:</p> <ul style="list-style-type: none"> • Secured access • Decontamination of all waste on site • Decontamination of laboratory clothing before laundering • Baseline serum (if applicable) | <p>Primary Barriers:</p> <ul style="list-style-type: none"> • Class II biosafety cabinet or other physical containment devices used for all open manipulation of agents. • PPE: Protective laboratory clothing, double gloves, respiratory protection as needed | <p>BSL2 Plus:</p> <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double-door access • The exhaust air not recirculated, and HEPA filtered. • Negative airflow into lab required |

| | | | |
|---|---|--|--|
| 4 | BSL3 Practices Plus: <ul style="list-style-type: none"> • Clothing change before entering. • Shower on exit • All material decontaminated on exit from facility | Primary Barriers: <ul style="list-style-type: none"> • All procedures conducted in Class III BSCs or Class II BSCs in combination with full-body, air-supplied, positive-pressure personnel suit | BSL3 Plus: <ul style="list-style-type: none"> • Separated building or isolated work zone. • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements |
|---|---|--|--|

Source: BMBL, 6th Edition

Biosafety in Microbiological and Biomedical Laboratories (BMBL) also describes Animal Biosafety Levels (ABSL) for the use of research animals. More detailed information on Biosafety Levels is available on the CDC website: <https://www.cdc.gov/labs/BMBL.html>.

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 presenting minimal potential hazard to laboratory personnel and the environment (e.g., *E. coli* for cloning, *Saccharomyces cerevisiae*, common soil bacteria). 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.

Nonetheless, standard microbiological practices must be met—no food and drink in the lab, proper lab attire (closed-toe shoes, long pants), and hand washing and eyewash capacity. 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. However, PPE should be considered at BSL1 based on a risk assessment of the chemical, physical, or radiological hazards present and used in the lab.

Biosafety Level 2 builds upon BSL1. BSL2 is suitable for work involving agents associated with human disease and that pose moderate hazards to personnel and the environment. It differs from BSL1 in the following ways: 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.

BSL2 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 diseases 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 Cal/OSHA BBP Standard for specific required precautions. Infectious agents or material likely to contain infectious agents listed by the Cal/OSHA ATD Standard are likely to require BSL2 containment with the use of dedicated safety equipment in order to prevent aerosol exposure.)

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. PPE such as splash shields, face protection, lab coats or gowns, and gloves should be used as appropriate.

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

C. PRACTICES AND PROCEDURES

The following practices, corresponding to BSL2, are important for the prevention of laboratory infection and disease and 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 an RG3 organism, contact the EH&S Office at x6727 for additional information.

1. Personal Hygiene

- Do not eat, drink, chew gum, use tobacco, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
- Do not store food for human consumption in laboratory refrigerators or cold rooms.
- Wash hands frequently after handling infectious materials, after removing latex/nitrile gloves and protective clothing, and always before leaving the laboratory.
- Keep hands away from your mouth, nose, eyes, face, and hair.
- Restrain long hair so it cannot contact hands, specimens, containers, or equipment.
- Do not remove PPE (such as cloth lab coats) from the lab.
- First-aid kit(s) should be available, fully stocked, and current (not expired).

2. 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, drinking, or smoking.

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.

3. Hand Washing Technique

Based on the 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.

4. Using Hand Sanitizer

Based on the 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 hand 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, including *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.

D. LABORATORY PROCEDURES FOR HANDLING INFECTIOUS MICROORGANISMS

- A. This Biosafety Manual outlines general activities and defines standard or safe operating procedures (SOP). In most cases, your lab's approved IBC protocol and this Biosafety Manual will provide the necessary information to work safely. However, the IBC may require laboratories to provide an additional dedicated Laboratory Biosafety Manual to describe particular research material, outline specific laboratory activities, and define custom laboratory SOPs.
- B. If you are working with recombinant DNA and/or biological material at BSL1 or higher, you must obtain approval from the [Caltech IBC](#). The IBC Administrator can be reached at x6435 or by email at ibc@caltech.edu.
- C. Principal Investigators (PIs) and/or designated laboratory safety coordinators 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 agent(s) in use and the appropriate emergency contact personnel. Templates of these biohazard signs will be generated by the EH&S Office based upon the information provided in your lab's IBC Protocol. Contact the EH&S Office at x6727 or safety@caltech.edu.
- G. BSL1 and BSL2 laboratories must have a sink for hand washing, have an eyewash station that is tested/flushed monthly, be relatively clutter-free, and be easy to access.
- H. Wear a fully fastened laboratory coat and protective gloves when working with infectious agents or whenever handling potentially hazardous materials, including human blood and body fluids.
- I. Remove and leave all protective clothing, including gloves, within the BSL2 laboratory before exiting. If transport of research materials through public spaces is required, one clean glove may be put on and the ungloved hand used to handle public equipment (e.g., door handles, elevator buttons).
- J. Never mouth pipette; use mechanical pipetting devices.
- K. Never sniff cultures.
- L. When practical, perform all aerosol-producing procedures such as shaking, grinding, sonicating, mixing, and blending in a properly operating biosafety 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.

- M. Centrifuge samples containing infectious agents in durable, shatter-resistant, closable tubes. Use a centrifuge with sealed heads or screw-capped safety cups, including the proper O-ring. After centrifugation, open the tubes within a BSC.
- N. Minimize the use of needles, syringes, razor blades, and other sharps when possible. After use, syringe-needle units must be disposed of in a dedicated sharps container without removing, bending, or recapping the needles.
- O. Wipe work surfaces with an appropriate disinfectant according to the corresponding IBC protocol before and 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 and other non-laboratory personnel 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.

E. CONSIDERATIONS FOR BIO-AEROSOLS

When manipulations of microorganisms or cell cultures present a potential to create aerosols, use a biosafety cabinet (BSC). Do not use a “clean bench”—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.**

1. Examples 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 microcentrifuge tubes in a standard microcentrifuge
- Vortexing liquid samples

2. How to Limit Aerosol 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/parafilm 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 biosafety cabinet or fume hood.

If the biological material used in the lab is known to harbor microorganism(s) that spread via droplets or aerosol and is(are) used in the laboratory with procedures anticipated or known to generate droplets or aerosols, the laboratory supervisor should ensure the lab is compliant with the Cal/OSHA ATD Standard.

F. SHARPS PRECAUTIONS AND DISPOSAL

NEVER RECAP NEEDLES BUT IF YOU MUST... USE ONE HAND!

Other sharps precautions include the following:

- 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 biohazard sharps container.
- **Never overfill biohazard sharps containers.** When the container is 2/3 full as indicated by the “full line” on the container, close it, and contact the EH&S Office for pick up by completing the online request form via the [Facilities Service Requests](#) 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 syringes 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 EH&S 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 when it must be done. If 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:**



First, place cap on a level horizontal surface; gently slide needle half-way into cap...

Then, slowly tip up needle end of the device and allow cap to slide over needle...

Finally, use the thumb of the hand holding the device to secure the cap on the syringe.

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, then secure it by firmly pushing the needle downward into the cap.

There are also commercial needle recapping devices available for this purpose.

Remember! Keep a designated sharps container nearby for disposal of sharps and do not recap unless absolutely necessary.

G. ENGINEERING CONTROLS

1. 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 BSO, in consultation with Design and Construction, reviews plans for laboratory design at BSL2 and above to ensure that adequate control measures are in place. The BSO is available for consultation on these matters.

2. Laboratory Ventilation (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 strongly recommended at BSL2, it is required at BSL3 or when handling material requiring BSL3 procedures.
- **To maintain negative room pressure, laboratory doors should be kept closed while biohazardous work is taking place.**

3. Biosafety Cabinets (BSC)

Biosafety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms and other biohazardous material. 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 any materials inside the cabinet from contamination from the lab environment.

4. BSC types

Two kinds of modern biosafety cabinets, designated as Class II and III, meet varying research and clinical needs. 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. **Hazardous chemicals must NOT be used in a Type A BSC.**
- **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% exhaust. **Because of the greater safety margin, small amounts of nonvolatile chemicals, carcinogens, or radioactive materials can be used in this cabinet.**

5. BSC Location and Certification

Since the air curtain created at the front of the cabinet can be easily disrupted, a BSC should be located away from air supply registers, entrances, high traffic areas, and laboratory equipment, e.g., centrifuges, that create turbulence – [for more details click here](#).

- A BSC used with biohazardous materials must be professionally certified after installation, annually, and after being moved.
- Caltech holds procurement contracts with three approved certifier Companies able to provide proof of NSF/ANSI 49 accreditation for each field technician sent to service BSCs at Caltech. Only an approved company can be used to recertify Caltech's BSCs.
- A BSC must be properly decontaminated before being moved or recertified.
- It is the Laboratory/Department's responsibility to contact their preferred BSC certification contractor for these services.

More details on the certification process for BSCs are available in the Caltech [Biosafety Cabinet Certification Quality Control Program](#) document.

Horizontal laminar flow clean benches are not biosafety 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 nor the environment.

Similarly, **chemical fume hoods** are not biosafety cabinets. They draw air in, potentially protecting the worker, but do not protect the material in the cabinet (your samples), and they 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.**

H. SAFE AND EFFECTIVE USE OF A BIOSAFETY CABINET

1. Start up

- Monitor alarms, pressure gauges, and flow indicators for any changes. Do not work in a biosafety cabinet while a warning light or alarm is signaling.
- 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 should be located away from high-traffic areas and doorways to common areas.
- Plan your work and place everything needed for the procedure inside the BSC.

2. 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 6 inches from the front BSC grill.
- Never place non-sterile items upstream of sterile items.
- Maintain the BSC sash at proper operating height, approximately level with your armpits.

3. 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 an elevation of lower than the window edge.
- Avoid rapid movement during procedures, particularly within the BSC but also in the vicinity of the BSC.
- 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, if 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.
- **NEVER 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 and constitutes a major fire hazard. 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.

4. Final Purging and Wipe-down

- After completing work, run the BSC blower for 2 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 a disinfectant appropriate for the agent in use.**

5. 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 (countertop, sides, and back wall). Investigators should remove their gloves and gowns or lab coat and wash their hands as the final step in safe microbiological practices. Refer to Chapter VI below for more information on disinfectants to be used.
- 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, 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. The drain pan should be wiped down with 70% alcohol to prevent corrosion.

6. Maintenance

- To function adequately and offer optimal protection, the cabinet airflow must be closely regulated, and the HEPA filters must be certified, and leak tested. Caltech requires all BSCs to be certified annually in accordance with NSF/ANSI 49. Caltech holds procurement contracts with three approved certifier Companies able to provide proof of NSF/ANSI 49 accreditation for each field technician sent to service BSCs at Caltech. Only an approved company can be used to maintain and recertify Caltech's BSCs.
- **Proper maintenance is imperative for BSCs intended for work at BSL2.**

I. AEROSOL-PROOF ROTORS AND SAFETY CUPS FOR CENTRIFUGES

Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes breaking during the centrifugation process. 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 3/4 full and avoid getting caps/closures wet.
- Wipe tubes down with a disinfectant after filling.
- **When following BSL3 practices, 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.

J. OTHER SAFETY EQUIPMENT FOR AEROSOL-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 BSC whenever possible.

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

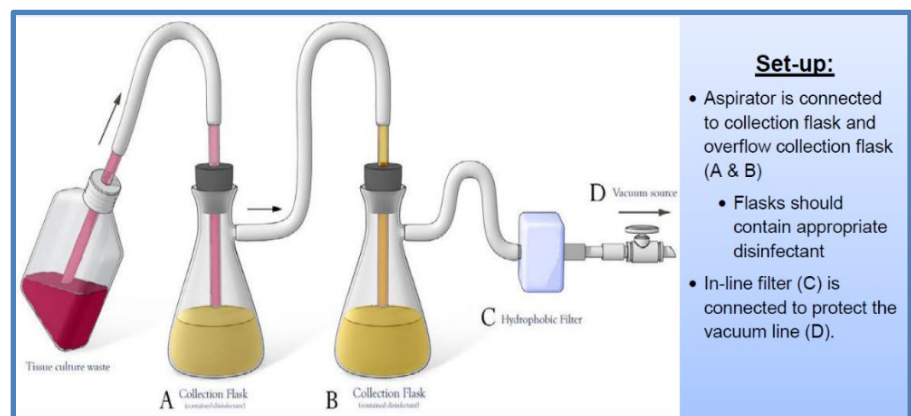
Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable loops and needles can be used.

K. Special procedures for Setting liquid waste containers:

• Vacuum Line Protection

When used in conjunction with vacuum lines, the vacuum lines must be protected from contamination:

- 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.



All Liquid traps should also be secured in leakproof secondary containers to avoid leakage on the lab floor.

- When setting up the first flask, **ensure that enough bleach is added to the empty liquid waste container before it is used for liquid collection so that there is a 10% final concentration of bleach solution.**
 - The presence of bleach in the waste receptacle will ensure inactivation of biological material immediately when it is collected in the waste container.
 - The amount of bleach added must be enough such that the final concentration is 10% bleach when the liquid waste reaches capacity.
- **Ensure that lids and/or caps are properly always secured on waste containers.**
- **When used in conjunction with liquid flow equipment (flow cytometer, cell counter, cell sorter, etc.)**
 - Use according to manufacturer instructions and keep original waste containers if possible.
 - Liquid waste containers should also be secured in leakproof secondary containers to avoid leaks on the lab floor.
 - When setting up the waste container, **ensure that enough bleach is added to the empty liquid waste container before it is used for liquid collection such that the final concentration is 10% bleach when the liquid waste reaches capacity.**
 - The presence of bleach in the waste receptacle will ensure inactivation of biological material immediately when it is collected in the waste container.
 - The amount of bleach added must be enough such that the final concentration is 10% bleach when the liquid waste reaches capacity.
 - Ensure that lids and/or caps are properly always secured on waste containers.



Moving liquid biohazardous waste containers:

- Always proceed with caution when moving liquid waste containers.
- Ensure the lids and/or caps are properly secured, and that the container destination is access free.
- Always place the liquid waste container in a designated sink or in a secondary container away from high traffic areas.

Disposing of liquid biohazardous waste containers:

- When a liquid waste container is almost at capacity, disconnect it from the equipment or vacuum line carefully and proceed with disposal:

If the container also contains hazardous chemicals, it must be processed as chemical waste (Chemical waste tag and EH&S pick-up).

If the container only contains biological material and bleach:

- In a designated sink, add fresh bleach to “top-off” the container to ensure adequate disinfection prior to disposal.
- Wait 15-20 minutes and dispose down the drain.
- Rinse with water and reset for waste collection.

L. PERSONAL PROTECTIVE EQUIPMENT (PPE)

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.**

PPE 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 EH&S Office online at safety@caltech.edu or by calling x6727.

The following PPE should be made available in laboratories operating at BSL2:

1. 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. Fluid-resistant lab coats must be used in situations where soaking or splashing with biohazardous materials 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 potentially 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 containers.
- **Lab coats must never 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.

2. 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, hypoallergenic 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.**
- Inspect and replace gloves if torn, punctured, or contaminated. Gloves that have spent time sitting in their box can become brittle and will break and tear easily—the box should be replaced immediately.
- **Never use 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

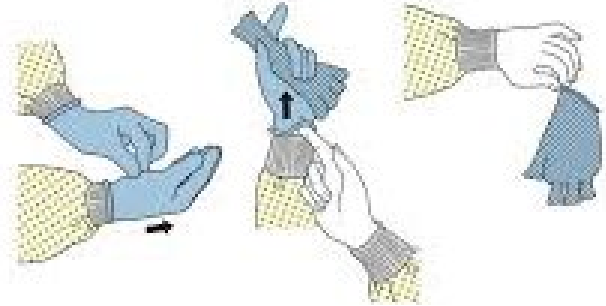
protection against hand contamination; thus, performing hand hygiene immediately after removing gloves is essential.

- 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.



Alternative Method for Glove Removal: The beak method

| "Beak Method" Glove Removal Steps | | |
|--|---|---|
|  |  |  |
| STEP 1: Using one gloved hand, pinch and pull the base of the other gloved hand. | STEP 2: Use the middle finger to scoop the cuff of the glove. | STEP 3: Pull the glove inside out over all the fingers and thumb to form a "beak." |
|  |  |  |
| STEP 4: With the beaked hand, pinch the opposite glove at the base and pull the cuff. | STEP 5: Roll the glove inside out and off the hand. | STEP 6: With the ungloved hand, use the index finger to pull the beaked glove off at the base of the beak and dispose into the appropriate waste container. Always wash your hands after glove removal. |

3. 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 PPE in the biohazardous waste bin.

4. Respiratory Protection

Engineering controls and work practice controls are used to reduce employee exposure to aerosol transmissible pathogens. However, when those controls are not sufficient (e.g., interaction with infectious patients or samples, collection and manipulation of human respiratory samples), respiratory protection is provided to employees in accordance with the [Caltech Respiratory Protection Program](#). 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 EH&S Office for more information.

5. Footwear and Dress Code

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



M. 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 general (Laboratory Safety Orientation) and specialized training elements (Biosafety Courses offered by the BSO) in support of **the most critical: the lab- or project-specific training elements provided by each PI or PI's designee.**

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.

1. Institutional Biosafety Training Program

Biosafety Principles (BSL1) Course: This training module is recommended 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*). This training is available as a self-paced online module and each individual can complete this course every three (3) years unless a significant change in legal or Institutional policy or safety guidelines dictates a shorter time interval. PIs can request additional

refreshers or subject-focused training for their groups.

Biosafety Principles (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 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 dictates a shorter time interval. PIs can request additional refreshers or subject-focused training for their groups.

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 dictates a shorter time interval. PIs can request additional refreshers or subject-focused training for their groups.

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. This module is available as a self-pace online module or in-person on demand. Each individual shall complete this course every three (3) years unless a significant change in legal or Institutional policy or safety guidelines dictates a shorter time interval. PIs can request additional refreshers or subject-focused training for their groups.

Cal/OSHA Bloodborne Pathogens Training: This training is required for all personnel listed on an IBC protocol that describes work with human cells, blood, tissues, or other potentially infectious material (OPIM). Each individual shall complete this course annually as stated in the Cal/OSHA Bloodborne Pathogens Standard (California Code of Regulations, Title 8, Section 5193). Initial and annual training provided by the BSO is documented in the MyLearn database. After the first instructor-led training, an online annual refresher course is made available.

Cal/OSHA Aerosol Transmissible Disease Training: This training is required for all personnel listed on an IBC protocol that describes work with Aerosol Transmissible Pathogens–Laboratory as defined in Appendix D of the Cal/OSHA Aerosol Transmissible Diseases Standard (California Code of Regulations, Title 8, Section 5199). Each individual shall complete this course annually as required by the Standard. Initial and annual training is provided and documented in the MyLearn database.

2. Laboratory-specific, Job-specific Training

Parallel to the Institutional Biosafety training, the **PI or PI's designee must provide laboratory-specific training** to new personnel on the location of emergency equipment and emergency response guides, type of experiments being conducted and associated risk, 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 must also maintain the laboratory-specific training documentation.**

The EH&S Office has designed a [training record form](#) that laboratories can adapt and use for recording their specific trainings.

CHAPTER V: BIOLOGICAL WASTE DISPOSAL PROCEDURES

Remember: *“It is not because your experiment is done, that the risk is gone”*

Biohazardous waste disposal regulations are designed to protect not only 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 State and**

local regulatory requirements.

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.

A. DEFINITIONS

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

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

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

- **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.

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

1. Solid Recombinant DNA/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 (rDNA) or organisms or cells containing recombinant DNA, laboratory personnel are responsible for autoclaving this waste before disposal in the regular trash. A temperature indicator (autoclave tape with 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 or time or if the temperature indicator did not turn black, the waste load should be re-treated prior to disposal.

Type of items (examples): recombinant *E. coli* or yeast Petri dishes, items contaminated with cell lines of animal origins, recombinant flies, or worms.

2. 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 **leakproof, hard-walled secondary containers** with tight-fitting covers. The secondary containers are labeled with the biohazard symbol and the word "Biohazard" on all visible sides.
- 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.
- On scheduled intervals, a commercial waste hauler removes the biohazardous waste from the storage area for treatment at a licensed facility.

EH&S-contracted personnel are responsible for transporting and storing BSL2 waste prior to off-site treatment. EH&S-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, waste is properly managed inside the red bins (e.g., no overflow, pipettes arranged in a way that reduces puncture risks), and EH&S-contracted personnel have been informed of the need to come into the lab to collect the waste.

Type of items (examples): Petri dish with culture of RG2 organisms, items contaminated with cell lines of human origin, any cell lines producing viral vectors.

C. PLASTIC SEROLOGICAL PIPETTES AND PIPETTE TIPS

Collection and Disposal

Plastic serological pipettes and plastic pipette tips cannot easily puncture skin; therefore, they are not considered sharps. However, since these materials may puncture through a biohazardous waste bag when collected with other solid biohazardous waste, e.g., flasks and tubes, they must be appropriately managed if they are used with biohazardous materials. The following options are available:

Small pipette tips:

Biohazardous pipette tips may be collected within the BSC in an old media bottle or other closable plastic receptacle. When the container is full, it is closed, and placed in the biohazardous waste bag.

Plastic serological pipettes (1–50 mL):

Plastic serological pipettes may be collected in the regular red bins with other type of BSL2 waste **with the clear understanding that practices must be in place to ensure that the pipettes will not slide sideways or in all directions in a manner that will increase the risk of bag puncture.**



They may be collected separately from other BSL2 waste type in a small step-can with appropriate dimensions to avoid sideways placement that may cause the pipette to puncture through a bag. Thus, “bundling” the long plastic pipettes in this manner prevents the plastic pipettes from poking through the bags.

Plastic pipettes can also be collected in horizontal container of the appropriate size, lined with a red bag to allow proper positioning and aggregation of the pipette waste.

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. When full, these sharps containers are collected by the EH&S team and processed as any other sharps containers.

Please contact EH&S for additional information at x6727.

D. SHARPS WASTE COLLECTION PROCEDURES

Biohazardous sharps waste describes items 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. **Syringes that are not equipped with needles MUST also be disposed in a sharps container to prevent disposal in a land field.**

Biohazardous Sharps Collection:

- Collect biohazardous sharps in a rigid, red biohazard sharps container harboring the biohazard symbol. These are available for purchase in the Caltech stockrooms or online.
- 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 (or filled to the manufacturer’s filled line mark), close it and contact EH&S for pick up by completing an online request via the [Facilities Service Requests](#) 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 containers with tight-fitting lids. 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.

E. TREATING LIQUID WASTE BEFORE DRAIN DISPOSAL

Most fluid waste, including human blood or infectious cultures **that have been decontaminated** appropriately, can be discarded by pouring into the sanitary sewer, followed by flushing with water. Care should be taken to avoid the generation of aerosols. **Liquid waste containing hazardous chemicals or radiological materials cannot be disposed of through the drain but must go through the appropriate hazardous waste stream.**

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

- Recommended PPE:
 - Lab coat
 - Latex or nitrile gloves
 - Safety glasses
- Approved Disinfectant:
 - Bleach, a sodium hypochlorite solution (NaOCl), is a broad-spectrum disinfectant that is effective against enveloped viruses (e.g., HIV, HBV, HSV), vegetative bacteria (e.g., *Pseudomonas*, *Staphylococcus*, and *Salmonella*), fungi (e.g., *Candida*), mycobacteria (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, prepared fresh.
- Contact time:
 - An appropriate contact time of sodium hypochlorite with liquid waste is 20 minutes. 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 1 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.

USE BIOHAZARD WASTE (RED BAGS/RED CONTAINERS) AND PICKUP SERVICES BY EH&S FOR BSL2 WASTE DISPOSAL

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

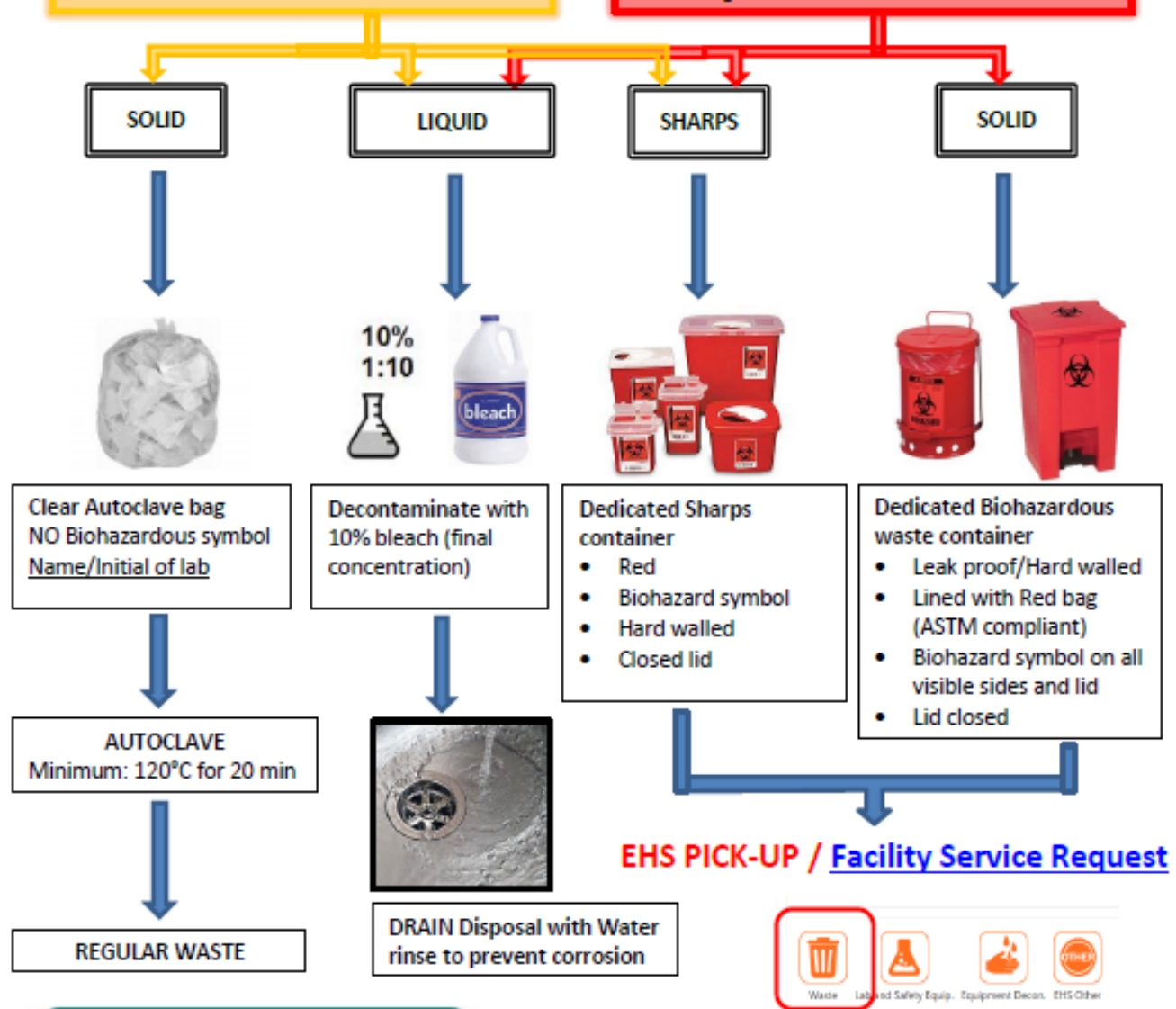
BIOLOGICAL WASTE DISPOSAL – DECISION TREE

NOT HAZARDOUS TO HUMAN HEALTH

- Animal (except Non-Human Primates) cell lines and tissues
- Recombinant DNA material
- Risk Group 1 agents containing rDNA (*E.coli*, *Yeast*)
- All Risk Group 1 agents

HAZARDOUS OR POTENTIALLY HAZARDOUS TO HUMAN HEALTH

- Human or Non-Human primate cell lines
- Human Specimens/blood
- Viral vectors (all state of replication competency)
- Risk Group 2 and 3 agents (pathogens)
- Biological Toxins



F. 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 EH&S 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 to make them safe for handling is achieved by disinfection or sterilization. Cleaning—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 EPA-registered antimicrobial products can only make efficacy claims against specific pathogens if 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:

| | Effective against | Dilution Shelf life | Contact time | Properties | Use |
|--|--|------------------------------|---|---|---------------------------------------|
| Bleach | Bacterial spores, vegetative bacteria, most viruses, fungi | 1:10 24 hours diluted | 10–15 min (surfaces) 20 minutes (liquid waste) | Inactivated by organic matter; corrosive; eye, nose, and 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 (e.g., Accel) | Broad spectrum of pathogens including norovirus and parvovirus | 1:40 3 months diluted | 5 minutes | Non-flammable, less corrosive than bleach | Surfaces in animal facilities |

A. BLEACH FACTS

- Useful for disinfection of waste before drain disposal.
- If used for surfaces, rinse 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% bleach), contain a stabilizer that extends shelf life up to 18 months.
- **Bleach is also a hazardous chemical (corrosive/oxidizer) and can react with other chemicals or heat. It must be stored in secondary containers and away from incompatibles.**
- **Always wear appropriate PPE when working with bleach.**

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 moving or servicing.

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

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 has contacted such cultures.
- Tissues, blood, and body fluids from humans and non-human primates.
- Tissues, blood, or body fluids from an animal that is carrying an infectious agent that can be transmitted to humans.
- Contaminated sharps.

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 them. 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.***
- 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 in water) 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 spill of known or potentially infectious agent.

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 most spills. One exception to this general rule is a spill of a significant volume outside of a biosafety cabinet or any untreated releases outside the laboratory facility. (Significance varies depending on the nature of the biohazard, but for purposes of this discussion, we define this to include culture volumes in excess of 3 liters.)

For spills of this nature:

- (1) Evacuate the area and post lab with "**DO NOT ENTER**" sign.
- (2) Call Caltech Security x5000 to secure the area and call the Safety Office x6727.

SMALL BIOHAZARDOUS SPILL OUTSIDE A BIOSAFETY CABINET (BSC)

1. Immediately stop all work and notify coworkers 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. **If you are properly trained in spill clean-up**, put on gloves and appropriate PPE—protective eyewear, lab coat, mask or face shield (splashing is likely to occur)—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, or broom and dustpan. **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 pickup and off-site disposal).
8. Remove all PPE into a biohazard (red) bag for appropriate disposal (EHS pickup and off-site disposal) and immediately wash hands.

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

You can adapt those instructions to your particular laboratory operation and print them to use as training and guidance documents in the laboratory.

**LARGE BIOHAZARDOUS SPILL (ONE TO THREE LITERS IN VOLUME)
OUTSIDE A BIOSAFETY CABINET (BSC)**

1. *Alert coworkers, 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 EHS Office (x6727) 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 PPE, i.e., lab coat, gloves, and mucous membrane protection (safety glasses and/or face mask).
7. Carefully pour an appropriate disinfectant solution* onto the towel-soaked spill; take care 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. Place paper towels and other contaminated waste into biohazard bag. Spray contaminated surface(s) 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 the EHS Office (x6727) if extra waste pickup is needed.
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 EHS Office if you have questions about the best disinfectant for your agent by calling x6727.

You can adapt those instructions to your particular laboratory operation and print them to use as training and guidance documents in the laboratory.

SPILL IN SMALL LABORATORY EQUIPMENT

Liquid spills on small laboratory equipment (centrifuge rotors, etc.) shall be contained as follows:

1. Don appropriate PPE (lab coat, gloves, safety glasses, mucous membrane protection).
2. Absorb excess liquid with paper towels.
3. Immerse the contaminated equipment in a freshly made 10% bleach solution and allow 10 minutes contact time, if possible.
4. Remove equipment from the decontaminant and 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 biohazardous waste.
7. Wash hands with soap and water.

SPILL IN LARGE LABORATORY EQUIPMENT

Liquid spills on large laboratory equipment (e.g., centrifuges, incubators, autoclaves) shall be contained as follows:

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

You can adapt those instructions to your particular laboratory operation and print them to use as training and guidance documents in the laboratory.

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 and/or Lab Safety Coordinator must ensure that all lab personnel know the location of the Guide and emergency equipment in the laboratory.

Caltech also has an Injury and Illness Prevention Program, and the EH&S Office conducts root cause analysis for 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.

Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation of an infectious agent should be given appropriate first aid. Any incidents, including life-threatening emergencies, should be reported immediately to Caltech Security Office (x5000).

Institutional SOPs for exposure response to live HIV, lentiviral vectors, and non-human primate materials (live or harvested) are available in laboratories handling these materials. These SOPs should be reviewed on a regular basis by all lab members using the material.

C. FIRST AID PROCEDURES

1. For a Needle Stick 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);
- Obtain medical attention immediately after washing the area; and
- Report the incident to both your PI and the EH&S Office.

2. For a Splash in the Eye

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

3. 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 at a safety shower or, if not available, a faucet;
- Obtain medical attention immediately after washing; and
- Report the incident to both your PI and the EH&S Office.

D. REPORTING INSTRUCTIONS

Report all injuries, accidents, near misses, and exposures to your direct supervisor and/or PI. In addition, ensure that all exposure incidents involving recombinant or synthetic organisms and infectious substances are reported to the EH&S Office and the BSO as soon as possible for follow up and notification of the appropriate regulatory agency, as necessary.

Any individual experiencing an exposure or potential exposure to biohazardous material will be offered 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 keep and review the HIV Exposure Response Procedure in the laboratory. Personnel exposed or potentially exposed to HIV or HIV pseudovirus must follow the posted procedures.

E. OBTAINING MEDICAL ATTENTION

https://safety.sites.caltech.edu/documents/19737/Incident_and_Injury_Reporting.pdf

CONTACT CAMPUS SECURITY x5000

| INFORM DISPATCHER OF... | CAMPUS EMERGENCY RESOURCES |
|--|---|
| <ul style="list-style-type: none">• The nature of the emergency• Your name• Phone number from which you are calling• Phone number where you can be reached• Your location <p>Unless there is an immediate threat to your safety, DO NOT hang up until you are sure no further information is required.</p> | <ul style="list-style-type: none">• Campus Security X5000 – 24 Hour Emergency Dispatch X4701 – Non-Emergency Assistance• Environment, Health and Safety X6727: 8:00 AM – 5:00 PM Mon – Fri X5000: 24 Emergency On-Call• Facilities Management X4717 – 24 Hour Service Center |

INCIDENT REPORTING PROCESS

- 1) EMPLOYEE, LAB COORDINATOR, PI, MANAGER, or SUPERVISOR** immediately reports any illness or injury to Campus Security by calling x5000 or 626-395-5000 **AND** notifies the/their direct report.
- 2) CAMPUS SECURITY** provides an initial field response, then reports all health and safety-related incidents to HR: leaveunit@caltech.edu and EHS: safety@caltech.edu.
- 3) PI's, MANAGERS, OR SUPERVISORS MUST SUBMIT** a completed [Supervisor's Injury Investigation Report](#) to Caltech's Disability and Leave Administration: leaveunit@caltech.edu as soon as possible, not to exceed three (3) days from when the actual incident/illness occurred.

RELATED RESOURCES

- [Medical Attention for Work-Related Injuries](#)
- [Supervisor's Injury Investigation Report](#)

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 2 years. Please contact the EH&S Office to receive 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 and are classified as unrestricted biological materials:

- Substances unlikely to contain pathogens, e.g., purified DNA, protein or antibodies, or cultures of RG1 microorganisms such as *B. subtilis* or *S. cerevisiae*;
- Neutralized or inactivated substances—Contact the EH&S Office to discuss proper means of inactivation;
- Environmental samples (food, water, soil, or dust);
- Blood for transfusion and 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/materials. Please contact the EH&S Office (safety@caltech.edu or x6727) for more information.

The following materials ARE regulated by DOT and IATA:

- **Category A infectious substance** is an infectious substance capable of causing permanent disability or 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 cultures of HIV or 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 that does not meet the criteria for Category A but is known or suspected to contain 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 primary 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.” – US 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 do not apply.

- **Regulated GMMOs** (genetically modified micro-organisms) 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. These include plants, fungi, bacteria, parasites, and animals (e.g., flies, worms).

If the GMMO is infectious or toxic, it must be sent as 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* or transgenic *Drosophila* lines.

An example 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 leakproof 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 leakproof plastic bag or container.
- The tertiary container is a rigid outer box of good quality with no dents or defects.
- Depending on the biological substance you are sending, various packaging tests may be required. Contact the EH&S Office for more information (safety@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—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.

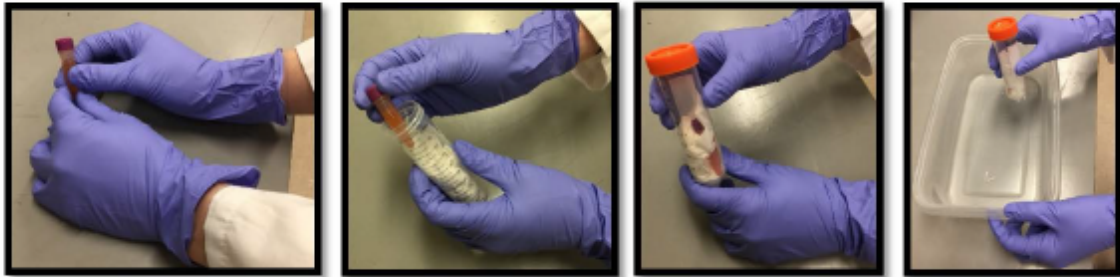
If you need to transport your sample outside of campus in local areas—Contact the EH&S Office for more information (safety@caltech.edu or x6727).

TRANSPORTING SAMPLES, BEST PRACTICES

- Laboratory gloves – including new gloves - should never touch door handles, elevator buttons, phones, card swipes, or any surfaces outside of the laboratory area.
- Disposable laboratory gloves (nitrile, vinyl or latex) should never be washed or re-used.

Best Safety Practices recommend:

- When transporting hazardous material outside of the laboratory:
 - Use a combination of sturdy, leak-proof primary and secondary containers with an outer package.



- The outer package is handled without gloves.



Only allowed alternative practice:

- ONE GLOVE RULE
 - Keep an un-gloved hand to touch surfaces outside the lab (door handles, elevator buttons)...
 - ...while holding your sample, in secondary containment, with the gloved-hand.



www.safety.caltech.edu – (626)-395-6727 – safety@caltech.edu
www.ibc.caltech.edu – (626)395-4699 - ibc@caltech.edu

D. IMPORTING BIOHAZARD MATERIAL IN THE US

1. CDC Import Permit for Infectious Agents

The CDC Import Permit Program (IPP) regulates the importation of infectious biological agents, infectious substances, and vectors under USPHS 42 CFR §§ 71.54, final rule February 2013. The IPP may inspect the facility before issuing a permit. Please note that obtaining previously imported material from a colleague within 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 BSO with questions or assistance with the permitting process or go to <https://www.cdc.gov/cpr/ipp/etool.htm>.

2. Material Requiring CDC Import Permits

- 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) requires 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 diseases.
- 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 <https://fwsepermits.servicenowservices.com/fwse>.
- 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 Macacine Alphaherpesvirus 1 (Herpes B virus).

Please note that the described material may also 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 <https://www.aphis.usda.gov/aphis/ourfocus/importexport>.

3. Material NOT Requiring CDC Import Permits

- Diagnostic specimens not known or suspected by the importer to contain an infectious biological agent that are 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 when 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 when 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.),
 - 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).

4. 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: <https://www.cdc.gov/cpr/ipp/eipp.htm>

5. 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 EH&S Office for help with this process. Information on APHIS permitting requirements is available at <https://www.aphis.usda.gov/aphis/resources/permits>.

The US Food and Drug Administration (FDA) requires a permit or registration before importation of food (except most meat and poultry), drugs, biologics, cosmetics, and medical devices into the US. See <https://www.fda.gov/industry/import-program-food-and-drug-administration-fda> 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. In addition, you must complete a [Caltech International Shipment Form](#) before exporting any material. Information may be obtained from Caltech's [Office of Export Compliance](#) website and the Department of Commerce Bureau of Export Administration at 202-482-0896 or online at <https://researchcompliance.caltech.edu/compliance/export>. Call x2641 or email exportcompliance@caltech.edu to speak with the Institute Export Compliance Office.

F. INACTIVATION OF BIOLOGICAL MATERIAL

Infectious material (cells, bacteria, viruses, etc.) can be treated using various methods to be inactivated. Inactivation is intended to retain characteristics of interest for the material (nucleic acid, proteins, membranes, organelles) while reducing or eliminating transmissible and/or infectious potential for the material.

Types of inactivation methods may include, alone or in combination:

- Physical (heat, ionizing irradiation, UV light);
- Chemical (chaotropic compounds such as guanidine hydrochloride, oxidizers such as chlorine and hydrogen peroxide)

- Natural antimicrobial strategies (enzymes such as lysozymes and virolynsins or antimicrobial peptides)

When choosing an inactivation method, several factors need to be considered including specific controls; the balance between efficacy of inactivation vs. the retention of desired characteristics; and the appropriate safety margin (i.e., overkill amount).

The *Biosafety in Microbiological and Biomedical Laboratory (BMBL)* – 6th edition, Appendix K, provides detailed information on inactivation methods efficiency and protocols.

The possibility that the inactivation and/or fixation treatment is incomplete, ineffective, or poorly executed should always be considered, especially when such samples are processed to be received from/sent to other organizations.

When receiving inactivated material:

- If planning on receiving and working with **inactivated or fixed samples of human or animal cells (not infected with pathogens) or RG1 or RG2 organisms**, IBC approval is not needed; however, it is best practice to ensure that the methods or treatments used to inactivate the biological material of interest have been verified and/or validated by the Institution, laboratory, or vendor providing you with the samples.
- If planning on receiving and working with **inactivated RG3 organisms, novel pathogens (e.g., SARS-CoV-2), or cells infected with those organisms**, IBC approval is required prior to receiving the material. Documentation of the methods or treatments used to inactivate the material of interest, data on specific batches/lots, and method verification and/or validation from the Institution, laboratory, or vendor providing the samples must be made available for review by the Caltech IBC.

When shipping inactivated material to other Institutions:

Verifying viability or infectivity (each sample/lot/batch) or using an experimentally validated method of inactivation is necessary to ensure that infectious organisms within the sample(s) are truly unable to grow, replicate, and/or generate infectious diseases.

- **The IBC requires that material and samples likely to contain RG2 or RG3 agents and novel pathogens are verified for efficient inactivation prior to being shipped to other institutions. Alternatively, inactivation can be achieved using an experimentally validated inactivation method. All processes must be described in the laboratory IBC protocol and approved by the IBC.**
- **The same requirements must be met to lower the containment level recommended for work with biological material within a laboratory or when transporting inactivated samples to other Caltech laboratories (PI-led or Core Facilities).**

CHAPTER IX: VIRAL VECTORS BIOSAFETY

Viral vectors are now very commonly used tools for cellular and molecular biologists, biological engineers, and neurobiologists. The use of viral vectors systems (both *in vitro* and *in vivo*) presents unique occupational health and safety challenges. Accidental exposure can occur in the laboratory through direct contact with the material (viral particles, freshly transduced cells, research animals); inhalation of concentrated aerosolized materials; droplet exposure of mucous membranes of the eyes, nose, or mouth; ingestion; or direct injection.

An in-depth understanding of the molecular mechanisms and strategies used for creating and using viral vectors is essential to conduct a risk assessment and implement adequate control measures necessary

for the safe handling of these agents.

The first step for viral vector risk assessment is the understanding that viral vectors originate from viruses that have been genetically modified to lose or retain specific viral functions. **In general, virulence attributes and the ability to replicate have been stripped from the viral vector genome**, preventing viral vectors from completing a regular virus replication cycle. However, engineered viral particles, by design, are expected to retain the ability to i) infect (enter and add genetic material) a host cell, ii) potentially interact with the host cell genome, and iii) promote expression of the added genetic material.

The following is an overview of the viral vector features that need to be considered in order to evaluate the risk of a particular construct. For the following considerations, replication incompetency is assumed for all constructs—if you are planning to work with a replication-competent viral vector, contact the BSO.

A. VIRAL VECTOR TROPISM

Viral tropism is the ability of a virus to infect a particular cell type, tissue, or host. Viral tropism depends on specific viral features such as the capsid architecture for non-enveloped viruses or the presence of dedicated envelope proteins for enveloped viruses. The tropism of a viral vector follows the same principles with the addition that capsid or enveloped proteins can be genetically modified to impact (restrict or enhance) the viral vector tropism.

Ecotropic viruses and viral vectors are usually restricted to a specific animal species. If that host species is rodent, chicken, or other non-human animal, then the virus or viral vector does not pose a significant risk to human health since it cannot promote entry into human cells or tissues. Ecotropic viral vectors are usually handled at Biosafety Level 1.

If a virus is known to target human cells, exposure should be avoided, and Biosafety Level 2 practices are the minimum requirement for working safely with those constructs.

Amphotropic or pantropic viruses are viruses known to infect a broad variety of cells, including human cells. An amphotropic/pantropic viral vector must be considered able to enter a human cell, i.e., a researcher cell, upon exposure.

Research protocols will often rely on pseudotyping to ensure cellular systems, *in vitro* or *in vivo*, are targeted by viral constructs. The ability to genetically influence viral tropism by modifying the envelope protein displayed on virion particles can lead to safer (restrictive tropism) or riskier constructs, and researchers should be mindful when designing their viral vectors. When possible, the lowest risk tropism should be chosen and at minimum, researchers must understand their construct's tropism and the appropriate containment level for each.

The G protein from the Vesicular Stomatitis Virus (VSV-G) is often used to pseudotype lentiviral vectors and is known to be a very permissive envelop protein. VSV-G-pseudotyped viral vectors can enter almost every cell type even if cells are not metabolically active or actively dividing. This is a higher risk tropism.

B. IMPACT ON TARGET CELL GENOME INTEGRITY AND FUNCTION

Once they gain access to cells, viral vectors retain various abilities to interact with the host cell genome and can exert some influence over host cell function depending on the transgene they carry and express.

For viral vectors classified as “**non-integrating**” such as adeno-associated virus (AAV) or adenovirus, the main risk associated with each construct is linked to the nature and function of the genetic cargo delivered to the host cell and likely expressed in that cell. Despite not being designed to integrate into the host cell genome, chromosomal integrations are sometimes observed with AAV.

Retrovirus and lentivirus are, by design, **expected to integrate into the chromosome of their host cells**. This is a desired trait retained by researchers to allow persistence and transmissible expression of the newly integrated genetic information. However, a possible consequence of chromosomal insertions

upon accidental exposure to retrovirus and lentivirus is the dysregulation of the host cell cycle and the initiation of an uncontrolled tumorigenic or oncogenic process.

This mechanism is called **insertional oncogenesis** and has been extensively documented in mice exposed to murine retrovirus (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3186009/>). Even if very little data exist to directly link exposure to viral vectors and human tumor or cancer diagnosis, this is a risk to consider when designing and handling retrovirus and lentivirus.

In addition to the risk associated with the integration ability of a viral construct, the newly introduced genetic information also affects the cellular outcome upon exposure. The nature and function of each transgene is not always fully known nor understood, and genes impacting cellular regulation, immunogenic cascade, or cell death are inherently higher risk than reporter genes or transgenes for which expression is not anticipated in the host cell.

C. SURVIVAL OUTSIDE THE HOST CELL

Physiological features of the capsid and viral envelop for each virus species impact their ability to “survive” and remain infectious outside a host cell. When packaging, receiving, aliquoting, or using viral vectors in biological systems, *in vitro* or *in vivo*, it is critical to identify the proper disinfection and surface decontamination practices for each type of viral vector. In general, enveloped viruses (retrovirus and lentivirus; neurotropic virus) are less robust and not able to withstand outside a host cell for an extended amount of time. They are susceptible to most common disinfectants. In a laboratory setting, 70% ethanol is usually sufficient for regular surface decontamination.

Non-enveloped viruses lack that more fragile feature and only consist of a very sturdy viral capsid. This feature renders them resistant to the harsh conditions found outside of a host cell and can make those viruses hard to deactivate. As an example, rhinoviruses are known to remain infectious up to 18 days on surfaces such as doorknobs. Viral vectors such as AAV and adenovirus can last on work surface for more than a week and are not efficiently killed by regular 70% ethanol. Therefore, freshly prepared 10% bleach is required for effectively decontaminating work surfaces when working with non-enveloped viral vectors.

As for any other infectious agent, concentration, contact time, and freshness of the disinfectant must be carefully considered.

D. LABORATORY PROCEDURES

Larger scale operations directly correlate with an increased risk for exposure to biological material—viral vectors are no exception. When working with viral vectors, the scale and type of laboratory procedures should be evaluated carefully.

Viral vector packaging protocols often rely on the use of larger volumes of tissue culture cells; the concentration of newly formed viral particles is expected to be high; and virus purification often requires centrifugation, ultracentrifugation, and/or the use of needles and syringes. This cumulation of large volume, high concentration, aerosol-producing practices, and use of sharps increases the likelihood of accidental exposure and therefore contributes to making viral vector packaging a high-risk procedure.

When available, a strategy to avoid this high-risk packaging is to obtain packaged viral vectors from a commercial source. Vectors come into the lab in very small aliquots, at medium to high concentration, and are usually ready to use. The likelihood of an accidental exposure is greatly reduced because a very small amount of material is handled for a very brief procedure (administration to cells *in vitro* or injection to animals *in vivo*). All exposure risks are not eliminated, but the overall “use” of the viral vector presents a lesser risk than the packaging phase.

If sourcing pre-packaged viruses is not an option, researchers should take care to identify when they are in the viral vector packaging phase, use SOPs designed to reduce the risk of exposure, and receive appropriate hands-on training by an experienced colleague before initiating the procedure.

E. VIRAL VECTOR RISK ASSESSMENT TOOL

The following table summarizes the features that should be evaluated when conducting a risk assessment for a viral vector (VV) construct.

| VV Feature | Lower Risk | Higher Risk |
|---------------------------|--|--|
| Tropism | Does not allow human cell entry | Allows human cell entry |
| Integration ability | No chromosomal integration | Chromosomal integration > insertional oncogenesis |
| Environmental sturdiness | Easy to inactivate/kill | Hard to inactivate/kill |
| Transgene nature/function | Reporter gene | Oncogene/Immunomodulatory gene/toxic genes SARS |
| Lab Procedures | Commercially sourced (low volume/low manipulation) | In-lab packaging |

F. MOST COMMONLY USED VIRAL VECTORS

1. Adeno-Associated Virus (AAV)

Adeno-associated virus (AAV) is a very small, non-enveloped virus with a single-stranded DNA genome. AAVs can mediate entry into human cells. They usually stay episomal (in the target cell cytoplasm) but can integrate into host chromosomes at low frequency.

AAVs are NOT susceptible to 70% ethanol, and a freshly prepared 10% bleach dilution must be used for disinfection.

Work with AAV viral vectors should be performed following BSL2 containment and practices.

2. Lentivirus

Lentiviral vectors are derived from HIV, which is an icosahedral, enveloped virus of approximately 100–110 nm diameter with a single-stranded, linear, positive-sense RNA genome. Upon entry into the host cell, retroviral RNA is converted to DNA by a virally encoded reverse transcriptase enzyme, and the cDNA product is integrated into the host's chromosomal DNA.

VSV-G pseudotyped lentiviruses are able to mediate entry into human cells and readily integrate into human chromosomes, which could cause insertional oncogenesis and other adverse effects if hazardous genes (such as oncogenes) are carried by the viral vector.

Lentiviruses are susceptible to common disinfectants: 70% alcohol (ethanol or isopropanol) and freshly made 10% bleach are appropriate.

Work with lentivirus should be performed at BSL2 following BSL3 practices.

| BSL3 Practices |
|--|
| no glassware—only plastic |
| Disposable PPE: Gown (high volume/packaging/higher risk procedures) Double gloving when using sharps |
| No open bench work—100% operation inside BSC |
| Centrifuge with aerosol tight lid—check the O-rings |
| SOP – dedicated for enhanced containment practices Work practices (PPE, Centrifugation, BSC, Specific processes) Onboarding/Training Emergency Response |

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 BSL2 with BSL3 practices.

Please contact the BSO for more information at x6727.

3. 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. Retroviral 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 that express glycoproteins derived from other enveloped viruses (usually can infect human cells). The most common glycoprotein currently used is VSV-G; however, newer pseudotypes are being derived from viruses such as SARS-CoV-2 (COVID), measles (Rubeola), Ebola, and Marburg. The VSV-G envelope allows infection in a wide range of mammalian (including human) and non-mammalian cells.

Retroviruses are **susceptible to common disinfectants**: 70% alcohol (ethanol or isopropanol) and freshly made 10% bleach are appropriate.

Work with amphotropic retrovirus should be performed following BSL2 containment and practices.

4. Neurotropic Viral Vectors

Certain species of viral vectors have a greater ability to promote entry and transduction of neuron cells and are tools favored by neurobiologists. These vectors have specific features that impact their safe usage in a laboratory setting.

Herpes Simplex Virus (HSV) Amplicon

Wild-type HSV can infect a broad range of cell types (dividing and non-dividing) in culture, and the wild-type virus only naturally infects humans. The recombinant HSV-1 (rHSV) amplicon has been rendered replication-incompetent via deletion of ICP4 and/or ICP27 genes and can only replicate in cell lines that provide the deleted gene in *trans*. rHSV can mediate cell entry into neurons and is often used to carry large recombinant DNA cargo. However, transduced cells do not have a long life span because transduction with rHSV is often cytotoxic.

rHSV is susceptible to common disinfectants: freshly made 10% bleach or 70% alcohol (ethanol or isopropanol) are appropriate.

Work with rHSV should be performed following BSL2 containment and practices.

G-Deleted Rabies Viral Vectors

Rabies viral vectors are enveloped viruses with a single-stranded, negative-sense RNA genome. They can infect neurons regardless of the species, but the modified G-deleted rabies viral vectors are unable to spread beyond the first infected neuron cell. Pseudotyping with EnvA/B further reduces the tropism of the rabies viral vectors such that they can only infect TVA/B expressing neurons, which is only naturally present in avian cells.

Rabies viral vectors are susceptible to common disinfectants: 70% ethanol and freshly made 10% bleach are appropriate.

As rabies is a negative-strand RNA virus with no DNA phase during its life cycle, recombination events that could restore the full virulence of the rabies virus are extremely unlikely.

Pre-exposure immunization and/or post-exposure prophylaxis exist for the wild-type rabies virus and are used to prevent rabies infection in humans. Depending on the design, these countermeasures can be recommended upon exposure to rabies viral vectors. Contact the BSO for additional information.

Work with G-deleted rabies viral vectors should be performed following BSL2 containment and practices.

G. GENOME EDITING TECHNOLOGY

The recent rise of gene editing technologies (TALEN, ZFN, CRISPR) has introduced new risk assessment challenges. CRISPR is now a simple, robust, and efficient tool to perform genetic engineering and is used in laboratory research on many varieties of microorganisms, eukaryotic cells, plants, and animals. Not only has this easily adaptable tool allowed for studies to determine gene functions, such as gene knockout or knock-in studies (where a gene is added to a genome), but it allows genome-wide screening, activation or repression of gene expression, and gene drive.

A gene drive propagates (or drives) genes into offspring at a higher inheritance rate than would be expected in nature in what is referred to as "super-Mendelian inheritance." Offspring not only inherit the modified gene, but they inherit the CRISPR system as well. The use of a gene drive should lead to more organisms with the gene (or disrupted gene) of interest—close to 100% inheritance instead of 50% of offspring as expected in Mendelian inheritance. Gene drive systems have been proposed for pest management, improvement of crop yields, and manipulation of vector populations to reduce the spread of diseases such as malaria. Gene drive experiments require robust risk assessment to evaluate the risk of environmental escape and should be carefully contained. The Caltech IBC must approve any gene drive experiment before it is initiated.

When used as a non-gene drive technology, CRISPR risk assessment requires evaluation of the anticipated function of the system as well as the potential off-target effects that can have major impacts on an organism.

The delivery methods used to introduce the CRISPR system into a cell (plasmid *versus* viral vector) directly impact the overall risk and containment requirements for a CRISPR project. CRISPR projects may be properly contained at BSL1 and/or ACL1 or may require higher containment such as ACL2 or even BSL2 and BSL3 practices. (Arthropod Containment Levels, or ACLs, are defined in Chapter XII.)

The Caltech IBC provides a [risk matrix for CRISPR projects](#). Please consult with the BSO for help with CRISPR risk assessment

CHAPTER X: BIOLOGICAL TOXINS

A. BASIC CHARACTERISTICS

Biological toxins are natural, poisonous substances produced as by-products of microorganisms (exotoxins, endotoxins, 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. However, mucous membranes of the eyes, nose, and mouth and breaks in the skin serve as portals of entry. Aerosol transmission, ingestion, and percutaneous transmission are also concerns 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 relatively unstable (destroyed rapidly when heated to $\geq 60^{\circ}\text{C}$). 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°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 activation of secondary messenger pathways (e.g., *Clostridium botulinum* and *C. difficile* toxins).

1. 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 biosafety 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 toxins are 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.

PPE should include a lab coat, gloves, and mucous membrane protection. All personnel in the lab should be trained in 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 table below.

2. Toxins That Require IBC Approval

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

Toxins noted in RED are considered Select Agents if being stored in large enough quantities (see Chapter XI). For more information, see <https://www.selectagents.gov/sat/permmissible.htm>.

B. SECURITY

Stocks of biological toxins must be maintained in locked cabinets, freezers, and/or refrigerators. Since biological toxins are not self-replicating, as are microorganisms, **maintaining an inventory or “record of quantity used” is highly recommended 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 EH&S Office upon request.

C. DECONTAMINATION METHODS

Most 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 |

^a 2.5% NaOCl ≈ 50% household bleach (1:2 dilution); ^b 1.0% NaOCl ≈ 20% household bleach (1:5 dilution); ^c 0.1% NaOCl ≈ 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 (except for T-2 mycotoxin, toxins are not dermally active). For significant exposures to biological toxins, contact both your PI and the EH&S Office and seek medical attention immediately.

CHAPTER XI: PRION-LIKE PROTEINS (PLP)

Prions refers to abnormal, pathogenic protein agents that are transmissible and able to induce abnormal folding of specific normal cellular proteins that are found most abundantly in the brain. The functions of these normal prion proteins are still not completely understood. Transmission of prions is thought to occur via direct contact with infected body fluids and tissues or via environmental contamination of soil, food and water.

Prion-like proteins (PLP) constitute a subset of modular polypeptides broadly expressed across different cell types and tissues and significantly associated with disease:

- Alpha-synuclein (Parkinson’s disease),
- Tau, beta-amyloid (Alzheimer’s disease),

- Tau, RNA-binding protein Fused in Sarcoma (FUS) (Frontotemporal lobar dementias),
- Polyglutamine-containing proteins (polyQ) (Huntington's disease),
- Superoxide dismutase 1 (SOD1); transactivation response element (TAR) DNA-binding protein-43 (TDP-43)
- FUS; Ubiquilin (ALS/Lou Gehrig's disease).

Prion-like behavior of these proteins or peptides include protein-based templated misfolding, the ability to propagate or spread along with neural systems (neuron to neuron with extracellular spreading). Additionally, these proteins have a fibrillar or aggregated form, often resistant to protease degradation, that has been shown to “seed” a pathology associated with a disease.

Given the overlapping features of proteopathic seeds between prion diseases and other neurodegenerative protein aggregation disorders, it is prudent to consider whether biosafety measures for research involving the use of these proteins, in their non-aggregated and aggregated forms, are sufficient. There is very little clinical data to suggest “infectivity” or “transmissibility” of the PLP; however, a better understanding of the cell-to-cell transmission process and the molecular behavior of the proteopathic seeds is needed to fully assess potential risk in a laboratory setting.

<https://academic.oup.com/jnen/article/79/11/1141/5912179>

Laboratories conducting experiments with the aggregated forms of these proteins, or any forms that has been shown to seed and propagate protein misfolding *in vitro* or *in vivo* must follow the principle of universal precautions and maintain BSL2 containment when working with the material. In addition, aerosol mitigation practices must be in place when inhalation exposure risk is identified.

Please consult with the BSO if you are planning to work with PLP.

CHAPTER XII: 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), the Administrative Committees on Biosafety (IBC and IRE), and the Administrative Committee on the Use of Human Embryos and Stem Cells.** The Office of Research Compliance also has responsibilities relating to responsible conduct of research, conflicts of interest, controlled substances, compliance with US 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 or 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 *NIH 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 *NIH 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 vertebrate and invertebrate animals. The *NIH Guidelines* mandate research institutions to form and administer an Institutional Biosafety Committee (IBC) to oversee at the Institutional level the use and safety measures associated with the use of recombinant DNA in research activities. Research involving recombinant or synthetic nucleic acids is covered under one of six sections (Sections III-A through III-F) of the *NIH Guidelines*. 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 highlighting the use of recombinant DNA and other biohazardous materials for IBC review.

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

The BSO conducts lab inspections, develops emergency and reporting procedures, conducts root cause analysis for lab accidents or near misses, reports recombinant and synthetic nucleic acid incidents and violations of the *NIH 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/>.

B. INCIDENT REPORTING TO NIH

The following incidents require **immediate reporting** to the NIH Office of Science Policy (OSP):

- Spills or accidents involving recombinant and synthetic nucleic acids covered under the *NIH Guidelines* that require BSL2 containment **and** resulted in an overt exposure, e.g., needle stick; splash in eyes, nose, mouth; or accidental aerosolization/inhalation.
- Spills or accidents involving recombinant and synthetic nucleic acids requiring BSL3 or BSL4 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 OSP **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 *NIH 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 do not need to be reported to the NIH. NIH OSP is available for consultation if there is uncertainty whether an incident is reportable. The incident report to NIH OSP 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 BSO or the IBC Administrator or see <http://ibc.caltech.edu/>.

C. SELECT AGENT REGULATION

The Federal Government has published a list of infectious agents and biological toxins that are strictly regulated due to their potential use as bioterror agents. Shipping, manipulation, and even possession of these “Select Agents” are heavily regulated at the Federal and Institutional level (3). **Currently, there are no viral or bacterial Select Agents approved for use on the Caltech campus.**

For more information about the Federal Select Agent Program, including a list of the agents that are currently regulated, please visit <http://www.selectagents.gov>.

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** toxins locked and maintain inventories to ensure the maximum exempted amount is not exceeded. Please contact the BSO 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 (Effective 02/21/2017) |
|---|--|
| Abrin | 1,000 mg |
| Botulinum neurotoxins | 1 mg |
| Conotoxins (short, paralytic alpha) | 100 mg |
| Diacetoxyscirpenol (DAS) | 10,000 mg |
| Ricin | 1,000 mg |
| Saxitoxin | 500 mg |
| Staphylococcal enterotoxins subtypes A–E | 100 mg |
| Tetrodotoxin (TTX) | 500 mg |
| T-2 toxin | 10,000 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 these items, please contact the EH&S Office at x6727.

D. 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 Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern](#) 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 DURC 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 DURC potential or that has been identified as DURC, and the Caltech DURC Committee (Institutional Review Entity, IRE).

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:

| SELECT AGENTS OR TOXINS | |
|---|---|
| Avian influenza virus—highly pathogenic | Marburg virus |
| <i>Bacillus anthracis</i> | Reconstructed 1918 Influenza virus |
| Botulinum neurotoxin* | Rinderpest virus |
| <i>Burkholderia mallei</i> | Toxin-producing strains of <i>C. botulinium</i> |
| <i>Burkholderia pseudomallei</i> | Variola major virus |
| Ebola virus | Variola minor virus |
| Foot-and-mouth disease virus | <i>Yersinia pestis</i> |
| <i>Francisella tularensis</i> | |

*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 ongoing 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; or
- Generate or reconstitute an eradicated or extinct agent or toxin listed above.

E. PI RESPONSIBILITIES

- Assess their 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 DURC;
- Ensure that their lab personnel are able to identify DURC and properly manage it (see https://researchcompliance.caltech.edu/documents/8678/durc_policy-october_2018.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.

F. FIVE 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 and 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 BSO at x6727 or the [Office of Research Compliance](#).

CHAPTER XIII: WORK WITH INVERTEBRATES, INSECTS, AND ARTHROPODS

Research experiments involving the use of invertebrates require facilities, equipment, trained personnel, and established practices that will ensure appropriate containment for the safety of the laboratory workers and the environment.

The review of research experiments involving invertebrates is essential to establishing the appropriate Invertebrate or Arthropod Containment Level (I/ACL) since different invertebrate species and different types of experiments will not necessarily pose the same level of risk for release in the environment and/or transmission of infectious material.

Risk assessment should take into consideration the species of invertebrate, their physical attributes (flying, crawling, swimming, burrowing, etc.), their natural distribution, their ability to act as vectors for disease transmission (diseases not limited to humans), and their potential impact on agriculture and native species.

When invertebrates, insects, or arthropods are genetically modified (transgenic animals) or when they are administered with microorganisms that have been genetically modified, the research falls under the Section III-D-4 of the *NIH Guidelines* and must be registered and approved by the Caltech IBC.

For help evaluating experiments using invertebrates, insects, or arthropods, contact the BSO at x6727

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Arthropod Containment

| Arthropod Containment Level (ACL) | 1 | | 2 | 3 | 4 |
|---|---|---------------------------------|---|---------------------------------|--|
| Arthropod distribution/fate in case of escape | Indigenous | Exotic but inviable if escaping | Exotic with potential to establishment (viable if escaping), transgenic vectors | | |
| Vector/Infectious status | Not a known vector Uninfected vector | | Infected with RG2/BSL2 material | Infected with RG3/BSL3 material | Infected with RG4/BSL4 material |
| Facility Features | <ul style="list-style-type: none"> Dedicated areas in laboratory – away from high traffic Closed doors and windows (screened if possible) Easily maintained work surfaces Escape traps, adapted to species | | ACL1 plus: <ul style="list-style-type: none"> Dedicated insectary with double door vestibule recommended, sealed electrical/plumbing, HVAC filtered and non-recirculating. All research/housing supplies should be stored in the dedicated space for arthropod work. Hand washing/shower equipment Access control | | Consult immediately with the Institute Biosafety Officer |
| Primary Barriers | <ul style="list-style-type: none"> Appropriate containers/cage/holding tubes that prevent escape at all life stages. Containers are cleaned on a regular basis | | <ul style="list-style-type: none"> Autoclaving or chemical decontamination of arthropod containers - at the point of use. Containers must be unbreakable (Disposable recommended) | | |
| Work Practices | <ul style="list-style-type: none"> All life stages of arthropod are killed before disposal (autoclave or freezing) Proper identification and labeling of containers Effective arthropod trapping program (traps and monitoring) Regular housekeeping Notification/signage (for vectors only) | | <ul style="list-style-type: none"> Autoclaving of infected arthropods prior to disposal Isolation of infected versus non-infected groups BSL2/ACL2 signage Training program with SOP designed to prevent escape (lab safety manual) Medical Surveillance program – if applicable Accidental release procedures (written, posted, drilled). PPE and respiratory protection – upon risk assessment | | |

All Arthropods administered with recombinant DNA material fall under section III-D-4 of the NIH guidelines and must be reviewed and approved by the IBC.

BIOSAFETY RESOURCES

American Biological Safety Association (ABSA): <https://absa.org/>

ABSA Biosafety Links: <https://absa.org/links/#bmb>

Biosafety in Microbiological & Biomedical Laboratories: <https://www.cdc.gov/labs/BMBL.html>

California Medical Waste Management Act: <https://www.cdph.ca.gov/Programs/CEH/DRSEM/CDPH%20Document%20Library/EMB/MedicalWaste/MedicalWasteManagementAct.pdf>

California Medical Waste Program: <https://www.cdph.ca.gov/Programs/CEH/DRSEM/Pages/EMB/MedicalWaste/MedicalWaste.aspx>

National Select Agent Program: <https://www.selectagents.gov/>

NIH Guidelines: <https://osp.od.nih.gov/biotechnology/nih-guidelines/>

NIH Office of Science Policy (OSP): <https://osp.od.nih.gov/>

NIH/OSP Frequently Asked Questions: <https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy#tab2/>

NIH/OSP IBC Information: <https://osp.od.nih.gov/policies/biosafety-and-recombinant-dna-policy/faqs-on-institutional-biosafety-committee-ibc-administration-may-2019/>

NIH/OSP Dual Use Research: <https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy#tab0/>

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