BIOSAFETY MANUAL

CHAPMAN UNIVERSITY
Orange, California

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LIST OF ABBREVIATIONS AND ACRONYMS

BSL	Biosafety level
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	Biological safety cabinet
BSO	Biosafety Officer
BUA	Biological Use Authorization
Cal/OSHA	California Occupational Safety and Health Administration
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DOT	U. S. Department of Transportation
EHS	Environmental Health and Safety
HEPA	High efficiency particulate air
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities
OPIM	Other potentially infectious materials
OSHA	U.S. Occupational Safety and Health Administration
PI	Principal Investigator
PPE	Personal protective equipment
rDNA	Recombinant DNA
USDA	U.S. Department of Agriculture

1.0 INTRODUCTION

This Biosafety Manual is part of Chapman University's Biological Safety program, which was established to accomplish the following goals:

- Protect personnel from exposure to potentially biohazardous materials.
- Prevent environmental contamination.
- Provide an environment for quality research while maintaining a safe workplace.
- Comply with applicable federal, state, and local requirements.

The Biosafety Manual provides safety guidelines, policies and procedures for the use and manipulation of biohazardous materials. Although the implementation of these procedures is the responsibility of the Principal Investigators (PI) and Laboratory Managers, its success depends on the combined efforts of all laboratory workers. Planning for and the implementation of biological safety must be part of every activity in which biohazardous materials are used.

PIs and Laboratory Managers are required to ensure that all employees and students in their respective work areas read this manual and have a working knowledge of its contents prior to working with biohazardous materials. This manual was designed as a supplement to meet the requirements of *Biosafety in Microbiological and Biomedical Laboratories*, 6th ed.

In general, the handling and manipulation of biohazardous materials such as biological agents and toxins, human and non-human primate materials, and synthetic and recombinant nucleic acid molecules, requires the use of various precautionary measures depending on the materials involved. This manual will provide assistance in the evaluation, containment, and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary. The Environmental Health and Safety (EHS) Office is available to assist.

2.0 REGULATIONS AND GUIDELINES

The following is a summary of the authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and toxins, and recombinant and synthetic nucleic acid molecules.

- National Institutes of Health (NIH): *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.* These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA (rDNA) molecules and organisms containing them. In 1974, a Recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. This document has been amended and revised many times. The last amendment was in April 2024.

- Centers for Disease Control and Prevention (CDC) and the NIH: *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*. In 1984, the CDC/NIH published the first edition of the BMBL. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1 – 4, which are recommended for working with a variety of infectious agents in various laboratory settings. While not a regulation, the BMBL has been revised several times and is commonly seen as the "gold standard" for Biosafety. Chapman University is using the BMBL (Sixth Edition, 2020) as the basis for this Biosafety Manual.

- California Occupational Safety and Health Administration (Cal/OSHA): *Bloodborne Pathogens Standard (California Code of Regulations, Title 8, Section 5193)*. This regulation addresses the occupational health risk caused by exposure to human blood and other potentially infectious materials and meets or exceeds the standard set forth by US OSHA. It includes a combination of engineering and work practice controls, personal protective equipment (PPE) and clothing, training and medical follow-up of exposure incidents, vaccination, and other provisions. Chapman University established a Bloodborne Pathogen Exposure Control Plan to protect employees from exposure to Human Immunodeficiency virus, Hepatitis B virus, and other bloodborne pathogens. You can request Chapman University's Exposure Control Plan from the EHS Office or the Biosafety Officer (BSO). The Plan is also published on the Chapman University website (https://www.chapman.edu/faculty-staff/environmental/biological.aspx)

- Medical Waste Management Act (California Health and Safety Code, Sections 117600 – 118360). This regulation establishes a program regulating the handling and disposal of medical waste. The rule mandates how producing facilities (generators of medical waste) must handle medical waste from the point at which it becomes medical waste, to the point of its ultimate disposal. This regulation covers solid and liquid biohazardous waste as well as sharps.

- Packaging, shipment, and transportation requirements for infectious substances, diagnostic specimens, and biologicals can be found in the link to the U.S. Department of Transportation (DOT) in the last chapter of this manual. In addition, the International Air Transport Association (IATA) has requirements that apply to certain biohazardous materials that are shipped via air.

- U.S. Department of Health and Human Services (HHS): Additional Requirements for Facilities Transferring or Receiving Restricted Agents. In 1996, HHS published a set of requirements that require facilities and institutions to be registered and approved in order to transfer or receive certain biological agents and toxins. A similar regulation issued by the U.S. Department of Agriculture (USDA) applies to an additional list of biological agents and toxins. The link to the current list of restricted agents and toxins covered under this rule can be found in the last chapter of this manual. Typically, restricted agents include highly pathogenic agents that would be handled at Biosafety Level Three or Four, which Chapman

University does not store or use. Certain biological toxins when utilized above established quantity thresholds may require registration.

- Importation permits are required for certain infectious agents, biological materials, and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine. In addition, the USDA Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms, or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms under Title 7 Code of Federal Regulations Part 340 (7 CFR 340). In addition, the U.S. Department of Commerce may require an export permit if shipping specific highly regulated materials to certain countries. Always check with the BSO before importing or exporting biohazardous or biological materials.

3.0 ROLES AND RESPONSIBILITIES

Chapman University conducts biological research at Biosafety Level 1 and Biosafety Level 2 only. Chapman University does not maintain BSL-3 or BSL-4 facilities.

Recombinant and synthetic nucleic acid research conducted at Chapman University is limited to NIH Guidelines categories permitted at BSL-1 or BSL-2 containment. Human gene transfer studies, deliberate transfer of recombinant DNA into human subjects, and environmental release of genetically modified plants are not conducted at Chapman University.

The Biological Safety Program has been developed to protect the interests and resources of Chapman University, its employees, contractors, students, visitors, and the public from unnecessary and potentially harmful exposure to biohazardous materials. For the requirements and procedures that are presented in this manual to be effective, Principal Investigators, laboratory staff, employees and students must work collaboratively with the EHS Office.

3.1 Chapman University

Chapman University is responsible for ensuring that all research and related activities are carried out in a safe and prudent manner and in full conformity with the provisions of federal, state, and local regulations. To fulfill this responsibility, the Chapman University EHS Office develops and implements policies that provide for the safe conduct of all research and related activities and that ensure compliance with the applicable regulations. The BSO develops a Biological Safety Program and works with employees and contractors engaged in work with biohazardous materials in the discharge of Chapman University's responsibilities under these regulations. In addition, Chapman University has:

- Established an Institutional Biosafety Committee (IBC) that meets the requirements set forth by the NIH for institutions engaged in activities that involve Recombinant or Synthetic

Nucleic Acid Molecules. Chapman University has extended the purview of the IBC to review all potentially biohazardous work, in line with current “best practices” for IBCs.

- Ensured appropriate training for the IBC chairperson and members, PIs, laboratory staff, employees and students regarding biosafety requirements, their implementation, and workplace safety.

  - Responsibility for training staff is delegated to the PI or Laboratory Manager. They are best suited to train personnel on specific work practices. The EHS Office can provide specific biosafety training resources and assistance.

  - The IBC is responsible for ensuring that the PI and Laboratory Manager have sufficient training.

- Required investigators whose activities are subject to the NIH Guidelines, Cal/OSHA Bloodborne Pathogen Standard, and other regulations to comply with the provisions of those regulations.

- Assigned the responsibility for determining the necessity for medical surveillance of research personnel to the IBC and EHS for further guidance.

3.2 Institutional Biosafety Committee

Chapman University has agreed to follow the procedures and practices established by the NIH Guidelines for an IBC. The IBC is comprised of at least five members that collectively have experience and expertise and the capability to assess the safety of activities and potential risk to individuals, public health, or the environment in accordance with the NIH Guidelines. At least two members, not affiliated with Chapman University (apart from their membership on the IBC) serve on the IBC. They represent the interests of the surrounding community with respect to health and protection of the environment. One member is a non-doctoral person from the laboratory technical staff. The committee must have a member with expertise in animal containment principles and one with expertise in plant containment issues. Additional *ad hoc* expertise may be obtained when necessary for protocol review. No member of the IBC is involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or expects to be, engaged or has a direct financial interest.

The IBC is responsible for reviewing all recombinant or synthetic nucleic acid molecule activities conducted at Chapman University for compliance with the NIH Guidelines, and approving those projects that it finds are in conformity with the Guidelines. In addition, the IBC will review protocols involving other biohazardous materials such as microbes, human and non-human primate materials, and biological toxins. This is done by submission of a Biological Use Authorization Application (BUA).

Activities that require the submission of a BUA include the use of

- Human materials including human cell lines and primary human tissues
- Microorganisms
- Recombinant nucleic acids
- Biological toxins
- Animal inoculation studies

This review includes:

- Providing an independent assessment of the containment levels required for the proposed activities.
- Evaluating facilities, procedures, and practices, and the training and expertise of personnel and notifying the PI of the results of their review.
- Developing emergency plans to cover accidental spills and personnel contamination resulting from such activities.

3.3 Environmental Health and Safety

The EHS Office administers the Biological Safety Program and other Chapman University safety programs. The BSO is responsible for implementing and overseeing the technical aspects of the Biological Safety Program.

3.4 Biosafety Officer

The BSO's duties include, but are not necessarily limited to:

- Providing technical advice to the IBC and researchers on laboratory containment and safety procedures.
- Overseeing annual inspections to ensure that compliance with standards, guidelines, and regulations are rigorously maintained.
- In cooperation with EHS, developing emergency plans for handling spills and personnel contamination.
- Reviewing project registrations.
- Working with the EHS Office on all aspects of the Biological Safety Program.

3.5 Occupational Health Services

When the IBC identifies projects that may require medical surveillance, the BSO will coordinate the services of an external medical provider. This provider may also be involved in the review of exposures to biohazardous materials if there is a laboratory-related injury or illness. An occupational health review may be required if there is:

- Work with human blood or OPIM
- Work with RG-2 pathogens
- Animal work involving biohazards

- Respirator use
- Exposure incidents

3.6 Principal Investigator

The PI is responsible for compliance with Chapman University Biosafety policies and procedures and for the safe operation of the laboratory. Their knowledge and judgment are critical in assessing risks and appropriate application of these Chapman University Biosafety requirements.

As part of this responsibility, the PI must ensure that:

- No work involving microbes, human and non-human primate materials, biological toxins, and recombinant or synthetic nucleic acid molecules or work with these materials in laboratory animals is initiated until it has met all the requirements established by Chapman University as outlined in this manual;

- The appropriate Risk Group classification of the microorganism is identified and that the appropriate microbiological practices and laboratory techniques and containment facilities are used in the proposed research;

- All significant violations of the policies and procedures and all significant research-related accidents (spills, needle-sticks, injuries, etc.) which result in overt or potential exposure to infectious materials **will be reported immediately** to the BSO and the EHS Office and must be reported to University Risk Management via the web-based incident Reporting Form found at this link: Significant exposures shall be reported promptly to the IBC.

- Methods for dealing with accidental spills and personnel contamination are available;

- Required permits such as those required by the USDA and/or the CDC for work with certain animal and plant pathogens are obtained;

- Appropriate importation, exportation and interstate shipping requirements for biological materials and dry ice are followed.

Prior to initiating research, the Principal Investigator will:

- Obtain appropriate IBC or BSO approval for work with biological materials, and provide updates when changes occur in the work such as new personnel or additional biological materials;

- In consultation with the BSO and an Occupational Health Physician who at the referral of EHS determines the usefulness of serological screening, the requirements of medical surveillance, and the availability of vaccinations, if necessary;

- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccination or serum collection;

- Make employees aware that the Hepatitis B vaccination series is available free of charge to the employee if the employee has the potential for occupational exposure to human materials;

- Ensure that personnel working with biological materials are appropriately trained so that they are aware of the hazards and are proficient in the practices and techniques required for the safe handling of such materials.

While conducting research, the Principal Investigator will:

- Supervise the performance of the staff to ensure that the required safety practices and techniques are employed;

- Investigate and report in writing to the BSO any significant biosafety problems pertaining to the pursuit of the research goals, specifically, new information which was not available at the time of the initial registration;

- Correct any conditions that might release biohazardous materials into the environment;

- Implement the procedures prescribed for dealing with laboratory accidents;

- Comply with all stipulations recommended or required by the IBC when his/her registration is approved.

3.7 Personnel

Research staff and students who work with biological materials must participate in all required biosafety training. In addition, they must work in a manner that is consistent with applicable regulations and the requirements listed in this Biosafety Manual, and follow all instructions from their respective Principal Investigator, Laboratory Manager, or Instructor.

4.0 TRAINING REQUIREMENTS

Biosafety training will be provided for all personnel working with biological materials. In addition, personnel who work with Risk Group 1 and 2 microbiological agents must have standard training in microbiological practices to ensure proper handling of the agent. Research staff and students working with Risk Group 2 agents must also have additional, project-specific training. It is the responsibility of the Principal Investigator, Laboratory Manager or Instructor to provide this

training. Project-specific training should include discussions about signs and symptoms of illness following an exposure to biological materials, potential hazards from exposure, and methods available to employees to prevent exposure.

Employees and students must be adequately trained prior to beginning any work with microbes, human source materials and other potentially infectious materials (OPIM), non-human primate materials, biological toxins and recombinant or synthetic nucleic acid molecules. Annual Bloodborne Pathogens training is required for all Chapman University employees with potential exposure to human blood, unfixed tissues and cells, and OPIM.

PIs are encouraged to review this Biosafety Manual with their employees and students and address the following topics:

- The biology of the microbes used in experiments or that may be in the materials used, with emphasis on potential biohazards;
- Good aseptic technique;
- Proper techniques for decontamination and disinfection;
- Emergency procedures;
- A review of all relevant safety practices, the potential hazards of the work, and what to do if there is a suspected or confirmed exposure to biohazardous materials.

All non-technical staff members such as but not limited to maintenance personnel are trained as necessary about workplace hazards based on their job function. This training, in most cases, familiarizes them with the potential hazards associated with biological materials in general. The responsibility for training contract employees belongs to the contractor, but the EHS Office is available to advise them. In cases where there is doubt or uncertainty about their personal safety, non-technical staff members should:

- Look for warning statements on doors, refrigerators, or other signage, such as the universal biohazard signs and avoid if at all possible areas posted with such signs;
- Be escorted into the area by a technical staff member.

5.0 DEFINITION OF BIOHAZARDS AND BIOLOGICAL MATERIALS

Biohazards are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. The risk can be direct through infection or indirect through damage to the environment.

Biohazards materials include:

- Certain types of recombinant or synthetic nucleic acid molecules
- Microbes that can cause disease in humans, animals or plants (e.g., parasites, viruses, bacteria, fungi, rickettsia and prions)

- Human and non-human primate materials that may contain infectious agents
- Biological toxins (microbial and non-microbial)
- Transgenic plants and animals

These materials may cause adverse health effects in other living organisms or cause significant impact to the environment or community if not used, stored or disposed of properly. The above materials may be used or stored in research or clinical laboratories, quality control laboratories, and teaching laboratories.

6.0 BIOLOGICAL MATERIALS THAT REQUIRE REGISTRATION AT CHAPMAN UNIVERSITY

The following biological materials require registration with either the Chapman University BSO or the IBC prior to use:

Table 1 Biohazardous Materials

Material	Approved By
Recombinant DNA / Synthetic Nucleic Acids	IBC
Microbes	IBC
Human Materials	BSO*
Non-Human Primate Materials	BSO
Biological Toxins	BSO*

* *May require IBC approval in addition to BSO approval if regulated or if requested by BSO.*

7.0 CLASSIFICATION OF MICROBES BASED ON RISK GROUPS

Risk Groups (RG) are the result of a classification of microbes based on their association with, as well as the severity of, disease in healthy adult humans.

The NIH and World Health Organization (WHO) have established four comparable classifications based on the pathogenicity of the microorganism and availability of effective treatment.

Risk Group 1
Agents are well characterized and are not associated with diseases in immunocompetent adults. These agents present minimal individual and community risk. An example of a RG1 agent is *Escherichia coli (E. coli)* K12.

Risk Group 2
Agents may be associated with serious human disease but are easily contained. Effective treatment and preventive or therapeutic interventions are often available. These agents present

moderate individual risk and low community risk. An example of a RG2 agent is varicella-zoster virus (chicken pox) and *Listeria monocytogenes*.

The Biosafety Officer can assist with the classification of a microbe. This information will be used when conducting the risk assessment.

8.0 RISK ASSESSMENT

Risk assessment is a process used to identify the hazardous characteristics of a material or process, the activities that can result in a person's exposure to the material or process, the likelihood that an exposure will cause an injury or illness, and the probable consequences thereof. Information identified by the risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent injuries or exposures.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and procedure hazards. In addition, the capability of the staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

A five-step approach is recommended:

1) Identify agent hazards and perform an initial assessment of risk including information on risk groups, infectious dose, route of transmission and environmental stability.
2) Identify procedure hazards including information on aerosol generation, sharps use, centrifugation and any animal work.
3) Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment.
4) Evaluate the proficiencies of staff regarding safe practices, medical considerations, training status and the integrity of safety equipment.
5) Review the risk assessment with the biosafety officer, subject matter expert, and the IBC.

The EHS Office and the BSO are available to assist in this process and should be contacted for questions concerning biological safety. Once a risk assessment is completed, the results should be communicated to everyone involved in the process. If necessary, written standard operating procedures (SOPs) should be established and distributed.

9.0 BIOLOGICAL SAFETY AND BIOSAFETY LEVELS

Biological safety or Biosafety is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure or injuries that result from working with biological materials. Biosafety includes the containment conditions under which

biohazardous materials can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the worker, persons outside of the work area, and the environment to potentially infectious agents. It can be accomplished by the following:

9.1 Primary Containment

Primary Containment offers protection of personnel and the immediate laboratory or work environment through good microbiological technique (practice) and the use of appropriate safety equipment such as biological safety cabinets and personal protective equipment.

9.2 Secondary Containment

Secondary Containment allows protection of the environment external to the laboratory or work area from exposure to biohazardous materials through a combination of facility design and operational practices. The combination of practices, containment equipment, and special laboratory or facility design can be made to achieve different levels of physical containment.

Currently, four Biosafety Levels (1-4) are recognized by regulatory agencies and define the level of primary and secondary containment necessary to protect personnel and the environment. Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Levels 3 and 4 (BSL-3, BSL-4) require specifically-engineered containment laboratories or facilities, which are not available at Chapman University.

Since all research at Chapman University is conducted at Biosafety Levels 1 and/or 2, this manual will focus on these two Biosafety Levels. A discussion of Biosafety Level 3 is included as additional information. For more information on higher containment level requirements, refer to the appropriate literature or contact the Biological Safety Officer.
The most important element in maintaining a safe work environment **is strict adherence to good microbiological and laboratory practices and techniques**. Everyone working with biohazardous materials must be aware of the potential risks. In addition, they must be trained and proficient in the practices and techniques required for handling such material.

Table 2 summarizes the four biosafety levels along with applicable primary and secondary barriers.

Table 2 Summary of Biosafety Levels (BSL-1 and BSL-2)

Biosafety Level 1 (BSL-1)	
Agents:	Not known to cause disease in immunocompetent adults.
Practices:	Standard microbiological practices.
Safety Equipment: (Primary Barriers)	None required.
Facilities: (Secondary Barriers)	Open benchtop: sink required.
Biosafety Level 2 (BSL-2)	

Agents:	Associated with human disease, hazards (exposure) include auto inoculation, ingestion, mucus membrane exposure.
Practices:	BSL-1 practice plus: Biohazard warning signs; “sharps” precautions; Biosafety Manual defining any needed waste decontamination or medical surveillance policies. Access to BSL-2 laboratories shall be restricted to authorized personnel who have completed required training and have been approved by the Principal Investigator or laboratory supervisor.
Safety Equipment: (Primary Barriers)	Primary barriers include Class II Biological Safety Cabinets (BSCs) or other physical containment devices used for all manipulations of agents that create splashes or aerosols of infectious materials; personal protective equipment (PPE): laboratory coats, gloves, face and eye protection, as needed.
Facilities: (Secondary Barriers)	BSL-1 plus: Acceptable method for disposal of waste.

Typically a laboratory or facility is considered to be operating at large scale if the volume of liquid in a single vessel or for a single experiment is equal to or greater than 10 liters. In general, it does not matter when that culture volume is reached (either in a roller bottle, bioreactor, or harvest vessel) upstream or as a result of a pooling operation downstream. Once a volume of greater than 10 liters is achieved anywhere in the process, the process is considered large scale. Biosafety levels for large scale operations are similar to what is described in Table 2, with additional emphasis on the larger volume of liquid and the necessary work practices and procedures for controlling exposures and for responding to spills or leaks.

10.0 BIOSAFETY GUIDANCE FOR SPECIFIC AREAS

10.1 Recombinant and Synthetic Nucleic Acid Molecules

As a condition for the performance of research with recombinant or synthetic nucleic acid molecules, Chapman University must ensure that work with these materials complies with the most current NIH Guidelines as well as state and local regulations. At Chapman University, the responsibility for ensuring that these activities comply with all applicable guidelines rests with the institution and the IBC acting on its behalf.

All recombinant or synthetic nucleic acid molecule research proposals require the PI to make an initial determination of the required level of physical and biological containment. For that reason, the NIH has developed six categories (III-A to III-F) addressing different types of research.

The following is a summary of the six (6) NIH classifications of rDNA projects and criteria for each category. Research falling under NIH Guidelines Sections III-A, III-B, or III-C is not permitted at Chapman University without prior institutional authorization and development of appropriate review and containment capabilities.

1) (III-A) requires IBC approval, Review by a Recombinant *DNA* Advisory Committee (RAC) and NIH approval before project initiation.

2) (III-B) requires NIH/OBA (Office of Biotechnology Activities) and IBC approval before project initiation.

3) (III-C) requires IBC and IRB (Institutional Review Board) approvals and NIH/OBA registration before project initiation. Approval by the Institutional Review Board does not replace IBC review requirements.

4) (III-D) requires IBC approval before project initiation:
   - Using human or animal pathogens (Risk Group 2) as host- vector systems;
   - Cloning DNA from human or animal pathogens (Risk Group 2) into non-pathogenic prokaryotic or lower eukaryotic host-vector systems;
   - Using infectious animal or plant DNA or RNA (ribonucleic acid) viruses or defective animal or plant DNA or RNA viruses in the presence of helper virus in tissue culture systems;
   - Experiments involving whole animals in which the animal's genome has been altered by introduction of rDNA or the use of those animals and require BSL-2 containment;
   - Experiments involving whole plants in which the plant's genome has been altered by introduction of rDNA or the use of those plants;
   - Experiments involving more than 10 liters of culture;
   - Experiments involving influenza viruses.

5) (III-E) requires IBC notice simultaneous with initiation.
   - Experiments involving the formation of recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus.
   - Experiments involving whole plants. (Other than those that fall under III-A, B, D or F)
   - Experiments involving transgenic rodents that can be done at BSL-1 conditions.

6) (III-F) exempt (But still require IBC review)
   - Experiments that are not in organisms or viruses.
   - Those that consist entirely of DNA segments from a single non-chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent;
   - Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or closely related strain of the same species) or when transferred to another host by well-established physiological means;
   - Those that consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria or plasmids (excluding viruses) when propagated only in the host (or closely related strain of the same species);
   - Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent;
   - Those that do not present a significant risk to health or the environment.

The Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research. The NIH Guidelines can be found at: https://osp.od.nih.gov/biotechnology/nih-guidelines/

Any PI in charge of a project with recombinant or synthetic nucleic acid molecules must sign and date a compliance statement on each registration form indicating his/her knowledge and intention to comply with the NIH Guidelines and to abide by the provisions of any applicable City/Town ordinances. This is included in the statement of responsibility in the Biological Use Authorization Application (BUA).

10.2 Animals in Research

The use of animals in research is subject to federal law (Animal Welfare Act) and state regulations. Chapman University complies with all federal and State and local regulations regarding animal use and care. For more information on Chapman University's policy on the use of animals, contact the IACUC Administrator at iacuc@chapman.edu. The use of biological materials in animals requires Chapman University Institutional Animal Care and Use Committee (IACUC) registration and approval.

10.3 Human Materials that May Contain Bloodborne Pathogens

In compliance with Cal/OSHA Bloodborne Pathogen Standard, Chapman University is committed to protecting its employees from risks associated with exposure to bloodborne pathogens (BBP) and has implemented a Bloodborne Pathogen Exposure Control Plan, which is managed by the EHS Office. The Exposure Control Plan may be obtained online (https://www.chapman.edu/faculty-staff/environmental/_files/bloodborne-pathogen-exposure-control-plan.pdf) or from the EH&S office..

As outlined in further detail in the BBP Exposure Control Plan, those classifications of employees that have a reasonable anticipated risk for occupational exposure to bloodborne pathogens need to be identified and included in the Bloodborne Pathogens Program.

Human cell lines, including commercially available established cell lines, shall be handled using Standard Precautions unless documentation demonstrates the absence of human pathogens, and the IBC or BSO has approved alternate handling requirements.

10.4 Biological Toxins

Biological toxins may be microbial or non-microbial in nature. Biological toxins are technically chemicals and are covered by the Chapman University Chemical Hygiene Plan. However, they should also be registered with the Biosafety Officer.

Examples of biological toxins include:

Microbial:	Pertussis toxin, Cholera toxin
Non-Microbial:	Ricin, snake venoms

Biological toxins should be handled with care and typically the practices and procedures used at BL-2 are appropriate for handling most biological toxins in a laboratory environment.

Note that some select biological toxins are highly regulated and subject to registration with the CDC or the USDA. These toxins must be registered with the IBC. In some cases limited quantities of a regulated biological toxin may be possessed per PI without the need for a CDC or USDA registration. Always check with the BSO prior to obtaining a new toxin to review the latest information on regulated toxins and permissible quantities. Information on regulated toxins may be found at http://www.selectagents.gov/.

10.5 Aerosol Transmissible Pathogens

Certain high-risk pathogens that can be transmitted via inhalation of aerosols are regulated under the California Code of Regulations. A full list of these pathogens can be found here: https://www.dir.ca.gov/title8/5199d.html. Before beginning any work with aerosol transmissible pathogens, researchers must contact the BSO for a risk assessment in addition to submitting an IBC registration. Chapman has implemented an Aerosol Transmissible Disease Exposure Control Plan, which is managed by the EHS Office and can be found at: https://www.chapman.edu/faculty-staff/environmental/_files/aerosol-transmissible-disease-exposure-control-plan-2020.pdf
Procedures likely to generate aerosols or droplets involving Risk Group 2 agents or potentially infectious human materials shall be performed in a certified Class II Biological Safety Cabinet unless an alternative containment method has been approved through risk assessment.

11.0 PRACTICES, PROCEDURES, ENGINEERING CONTROLS AND PERSONAL PROTECTIVE EQUIPMENT

11.1 Practices and Procedures

Infection occurs when disease-causing microbes enter the human body in sufficient numbers and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections (LAI):

- **Through the mouth (Ingestion)**—Eating, drinking and smoking in the laboratory; mouth pipetting; transfer of microbes to mouth by contaminated fingers or articles.

- **Through the skin (Percutaneous)**—Accidental inoculation with a hypodermic needle; cut with glass; open skin; cuts and scratches.

- **Through the mucous membranes of the eye or face (Mucous Membranes)**—Splashes of infectious material into the eye or face; transfer of microbes to eyes or face by contaminated fingers.

- **Through the lungs (Inhalation)**—Inhalation of airborne microbes.

Through the use of proper practices and procedures in the laboratory or work area, the above routes of exposure can be eliminated. The following procedures should be followed at all times:

- Always wash hands after removing gloves and before leaving the laboratory or work area.
- Do not touch your face with your hands.
- Use good aseptic technique to protect what you are working with as well as yourself and your coworkers.
- Do not eat, drink, or store food, beverages or medications in the work area.
- Do not apply cosmetics, eye drops or lip balm in the work area.
- Do not handle contact lenses in the work area.

11.2 Elimination and Substitution

Whenever possible a biohazard should be eliminated or replaced with one that is not as hazardous. For example, non-pathogen model systems should be used in place of pathogenic ones. Or later generation viral vectors which separate viral genes on separate plasmids should be substituted for earlier versions.

11.3 Administrative Controls

11.3.1 Universal Biohazard Symbol

A biohazard label is required for all areas or equipment in which biohazardous materials are handled or stored. The appropriate place for posting the label is in close proximity of the main entrance door(s) to laboratories and animal rooms, on equipment like refrigerators, freezers, centrifuges, incubators, and secondary transport containers. Labels, along with advice on where to post them, can be obtained from the BSO or the EHS Office.

11.3.2 Training

Good microbiological and laboratory practices are essential for a safe work environment. In addition to general laboratory safety training, all personnel should receive laboratory-specific training from their PI, Laboratory Manager or instructor.

Depending on the type of tasks being performed and the level of agents being used, some or all of the following additional controls/training may also be required:

11.4 Engineering Controls—Biological Safety Cabinets

Biological Safety Cabinets (BSCs) are designed to provide personnel, environmental and product protection when they are functioning correctly and the appropriate practices and

procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III have been developed. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems.

Biological safety cabinets must not be confused with other laminar flow devices or “clean benches”; in particular, horizontal flow cabinets which direct air towards the operator and should never be used for handling infectious, toxic or sensitizing materials such as but not limited to laboratory animals. “Clean benches” may be appropriate for certain procedures where product protection is necessary and the materials are non-hazardous such as for dust-free assembly of sterile equipment or media preparation.

Laboratory personnel must be trained in the correct use and maintenance of biological safety cabinets to ensure that personnel and product protection (where applicable) are maintained. It is highly recommended that all users of BSCs receive proper training in the care and use of this type of equipment. Before selecting any Biosafety Cabinet for purchase, contact the BSO and EHS office for a work specific assessment and selection criteria.

The correct location, installation, and certification of the biological safety cabinet are critical to its performance in containing biohazardous materials. All BSCs must be inspected at least annually and certified by trained and accredited service personnel according to the NSF (National Sanitation Foundation) Standard 49. Inspection and re-certification is mandatory if the cabinet is relocated or after major repairs, filter changes etc. To request service or certification, contact Facilities Operations or the BSO or EHS. CDC and NIH have published a guide on BSCs: Installation and Use of Biological Safety Cabinets.

11.4.1 Class I Biological Safety Cabinet

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

11.4.2 Class II Biological Safety Cabinet

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

11.4.3 Safe and Effective Use of Biosafety Cabinets

- Make sure the BSC is certified (NSF sticker) when it is installed or after it is moved, or repaired, and annually thereafter. Check the magnehelic gauge or electronic controls regularly for any indication of a problem;

- Understand how the cabinet works;
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking rapidly behind you, and opening and closing of laboratory doors may disrupt the airflow pattern and reduce the effectiveness of the BSC compromising product and personnel protection;
- Minimize the storage of materials in and around the BSC;
- UV lights should not be used if the BSC does not have a sash interlock to protect against accidental exposure. Contact the EHS Office/BSO for further information;
- Always leave the BSC running with the sash in the correct work position. If the sash is closed when the BSC is running, damage can occur to the motor-blower.
- Always wear PPE such as a laboratory coat and gloves. This provides protection to the worker as well as protects the product from unwanted contamination.
- Before using, wipe work surfaces with 70% alcohol or any other disinfectant suitable for the agent(s) in use. Wipe off each item you need for your procedures before placing it inside cabinet;
- DO NOT place any objects over the front air intake grille;
- DO NOT block the rear exhaust grill.
- Segregate contaminated and clean items. Work from “clean to dirty”;
- DO NOT use items with open flames. This creates turbulence in airflow and will compromise sterility; heat buildup may damage the HEPA filter and release of gas may result in explosion. A ceramic core heater may be used in lieu of a Bunsen burner for example;
- Move arms slowly when removing or introducing new items into the BSC;
- If you use a piece of equipment that creates air turbulence in the BSC (such as a micro-centrifuge, blender), place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating;
- Protect the building vacuum system from biohazards by placing a microbial filter between the vacuum trap and the source valve in the cabinet;
- Clean up spills in the cabinet immediately. Wait 10 minutes before resuming work;

- When work is finished, remove all materials and wipe all interior surfaces with 70% alcohol or any other disinfectant suitable for the agent(s) in use. Periodically clean under the front grille;

Remove laboratory coat, gloves and other PPE and wash hands thoroughly before leaving the laboratory.

11.5 Safety Equipment

For a comprehensive listing of safety equipment required in the laboratory refer to Chapman University's Chemical Hygiene Plan (CHP).

11.5.1 Safety Showers

Safety showers provide an immediate water drench of an affected person who may be exposed to biohazardous materials.

11.5.2 Eyewash Stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of biohazardous materials.

11.5.3 Ventilation Controls

Ventilation controls are those controls intended to minimize employee exposure to hazardous chemicals and biohazardous materials by removing air contaminants from the work site. There are two main types of ventilation controls:

- General (Dilution) Exhaust - a room or building-wide system that brings in air from outside and ventilates within. Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work.

- Local Exhaust or Filtration - a ventilated, enclosed work space intended to capture, contain and exhaust or filter harmful or dangerous fumes, vapors and particulate matter. In the case of hazardous chemicals, this includes a chemical fume hood. In the case of biohazardous materials, BSCs should be used.

11.6 Personal Protective Equipment

PPE is used to protect personnel from contact with biohazardous materials. Appropriate PPE may also protect the experiment or product from contamination. The following are examples of PPE that should be worn when handling biohazardous materials. In addition, follow all Chapman University requirements related to PPE for specific work locations such as laboratories. The instructor and/or PI is responsible for providing students with PPE, consistent with EHS guidelines.

11.6.1 Eye and Face Protection

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

11.6.2 Laboratory Clothing

This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated. The individual college/department is responsible for arranging for the proper cleaning of laboratory clothing using a vendor approved by EHS. All laboratory clothing used in the course of work in Biosafety labs shall remain in the labs and properly stored following use until retrieved for cleaning.

11.6.3 Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Medical grade latex or nitrile gloves must be worn when working with biohazardous material. Toxic substances, hazardous chemicals and other physically hazardous agents may require substance-specific gloves. Temperature resistant gloves must be worn when handling hot material or dry ice. Wash hands after removing gloves. For assistance in glove selection, contact the EHS Office.

Chapman follows the one glove rule in all labs. Gloves should never touch door handles, telephones, or computers. To touch these items, remove one glove and replace when you are done. Gloves should not be worn outside of research areas.

11.7 Safe Use of Laboratory Equipment

11.7.1 Pipettes and Pipetting Aids

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

- Always use cotton-plugged pipettes when pipetting biohazardous fluids;
- Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout”;
- Do not discharge biohazardous material from a pipette inside receiving container. Whenever possible allow the discharge to run down the container wall.
- Discard contaminated pipettes in an appropriately sized sharps container;
- When work is performed inside a BSC, all pans or sharps containers for contaminated glassware should be placed inside the cabinet while in use.

11.7.2 Syringes and Needles

Syringes and hypodermic needles are potentially dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. Safety-engineered sharps shall be used whenever feasible. Generally, the use of sharps should be restricted to procedures for which there is no alternative. When using syringes and needles with biohazardous materials:

- Wear appropriate eye/face protection (glasses, goggles, or face shields).
- Work in a Biosafety Cabinet.
- Wear gloves.
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid and bubbles from the syringe vertically into a cotton pad moistened with a disinfectant if applicable.
- Do not use a syringe and needle as a substitute for a pipette.
- Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe when possible.
- Use blunt-tip needles or safety-engineered sharps when possible.
- Needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe following use unless absolutely necessary.
- Always dispose of needle and syringe unit promptly into a Chapman University approved Sharps container.
- Do not overfill sharps containers (2/3 filled = full) and contact the college designated laboratory supervisor for pick-up and transfer into the waste room or facility. Contact EHS for questions on disposal schedules.

11.7.3 Cryostats

Frozen sectioning of unfixed human or non-human primate tissue poses a risk. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Cut-resistant gloves under disposable gloves may be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Wear appropriate eye/face protection (safety glasses, goggles, or face shield).
- Consider the contents of the cryostat to be contaminated and decontaminate frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use and if compatible with the equipment.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and carefully remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, or other infectious agents is cut.
- Handle microtome knives with extreme care. Stainless steel mesh gloves are recommended to be worn when changing knife blades.

- Consider solutions used to stain potentially infected frozen sections to be contaminated.

11.7.4 Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols.

- To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions.
- Users should be properly trained.
- Aerosols are also created by practices such as filling centrifuge tubes, removing supernatant, and resuspending sedimented pellets.

The greatest aerosol hazard is created if a tube leaks or breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings.
- Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc.
- Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC.
- Avoid overfilling of centrifuge tubes so that closures do not become wet.
- After tubes are filled and sealed, wipe them down with disinfectant.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs with filters.
- Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape.
- High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials.

- Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

11.7.5 Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small particle aerosols, which may contain viable microbes. The use of a shielded electric incinerator (ceramic core heater) or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended. Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence, which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

11.8 Housekeeping

Good housekeeping is essential to reduce risks and protect the integrity of biological experiments and products. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge.

Additional housekeeping concerns include:

- Keeping the work area neat and free of clutter—surfaces should be clean and free of infrequently used chemicals, glassware, and equipment.
- Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.

Old and unused chemicals should be disposed of promptly and properly. Refer to Chapman University's Hazardous Waste Management Program for more information.

Aisles and corridors shall be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, and avoidance of the creation of electrical hazards in wet areas. All laboratory equipment needs to be decontaminated and certified as being free of hazards before being released for repair, maintenance, or disposal.

11.9 Spill Procedures

Since spills of biological materials may happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazardous materials should have a basic biological spill kit ready to use at all times. For most instances, the basic kit can be assembled with materials already used in the laboratory. It is preferable to have the contents of the spill kit in one location.

11.9.1 Spill Inside the Work Area (Outside of a Biological Safety Cabinet)

- Clear spill area of all personnel;
- Wait for any aerosols to settle before entering spill area;
- Remove any contaminated clothing and place in biohazard bag for further processing;
- Don a clean gown or laboratory coat, safety goggles and gloves;
- Have a complete biological spill kit ready to go before you start the clean-up;
- Cover spill with paper towels or other absorbent material containing disinfectant;
- Encircle the spill with disinfectant (if feasible and necessary), being careful to minimize aerosolization;
- Decontaminate and remove all items within spill area;
- Remove broken glassware with forceps or broom and dustpan and dispose in sharps container. Do not pick up any contaminated sharp object with your hands;
- Remove paper towels and any other absorbent material and dispose in biohazard bags;
- Apply disinfectant to the spill area and allow for at least 20 minutes contact time to ensure germicidal action of disinfectant;
- Remove disinfectant with paper towels or other absorbent material and dispose of in biohazard bag;
- Wipe off any residual spilled material and reapply disinfectant before final clean-up;
- Wipe equipment with equipment compatible disinfectant (e.g., non-corrosive);
- Rinse with water if necessary;
- Place disposable contaminated spill materials in biohazard bags for autoclaving;
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving;
- Reopen area to general use only after spill clean-up and decontamination is complete;
- Inform all personnel and laboratory supervisor about the spill and successful clean-up as soon as possible;
- Fill out Incident report located in the Intranet. https://www.chapman.edu/faculty-staff/risk-management/reporting.aspx

11.9.2 Spill Inside the Biological Safety Cabinet

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

- Wear laboratory coat, safety goggles and gloves during clean-up;
- Allow cabinet to run during clean-up;
- Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 20 minutes contact time;
- Wipe up spillage and disinfectant with disposable paper towels;
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel;
- Discard contaminated disposable materials in biohazard bag(s) and autoclave before discarding as waste;
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving and further clean-up;

- Expose non-autoclavable materials to disinfectant, 20 minutes contact time, before removal from the BSC;
- Remove protective clothing used during cleanup and place in a biohazard bag for further processing;
- Inform all users of the BSC as well as the laboratory supervisor about the spill and successful clean-up as soon as possible.

11.9.3 Spill Inside a Centrifuge

- Have a complete biological spill kit ready to go before you start the clean-up;
- Clear area of all personnel and secure the area. Wait 30 minutes for aerosol to settle before attempting to clean up the spill;
- Wear a laboratory coat, safety goggles and gloves during clean-up;
- Remove rotors and buckets to the nearest biological safety cabinet;
- Thoroughly disinfect inside of centrifuge;
- Remove contaminated debris after disinfection, place in appropriate biohazardous waste container.

11.9.4 Basic Biological Spill Kit

- Disinfectant (e.g., bleach, prepare a 1:10 dilution when required);
- Absorbent Material (e.g., paper towels, spill pillows);
- Waste Container (e.g., biohazard bags, sharps containers);
- Personal Protective Equipment (e.g., lab coat, gloves, eye and face protection, booties);
- Mechanical Tools (e.g., forceps, dustpan and broom).

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations.

- As with any emergency situation, stay calm;
- Call Chapman Public Safety, if necessary, and proceed with common sense;
- Public Safety shall contact the EHS Office if further assistance is required, especially if the spill outgrows the resources in the laboratory.

11.10 Decontamination

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

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

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

- Heat
- Liquid Chemicals
- Vapors and Gases
- Radiation

Disinfection is normally accomplished by applying liquid chemicals. Vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) may be used for sterilization. Some liquid chemicals are also applied for sterilization, if used in the right concentration and sufficient incubation time. The following methods may be used and specific applications should be discussed with the BSO or the EHS Office.

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

- Liquid Chemicals Used as Disinfectants—The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved.

Variables to remember when disinfecting:

- Nature of surface being disinfected—Porous or smooth; the more porous and rough the surface, the longer a disinfectant may need to be in contact with the surface to be effective; Remember to liberally apply the disinfectant;

- Number of microorganism present—Higher concentrations require a longer application time and/or higher concentration of disinfectant;

- Resistance of microbes—Microbial agents can be classified according to increasing resistance to disinfectants and heat (refer to Table 3). Always select the disinfectant that will kill the microorganism(s) present;

- Presence of organic material—The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants. Cleaning of the surface prior to disinfection may be necessary;

- Duration of exposure and temperature—Increased exposure time may increase the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

Table 3 Increasing Resistance to Chemical Disinfectants

	Microbes	Examples
Least Resistant	Lipid Or Medium-Size Viruses	Herpes Simplex Virus Cytomegalovirus Respiratory Syncytial Virus Hepatitis B Virus Human Immunodeficiency Virus
	Vegetative Bacteria	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella cholerasuis*
	Fungi	Tricophyton sp. Cryptococcus sp. Candida sp.
	Non-Lipid or Small Viruses	Poliovirus Coxsackievirus Rhinovirus
	Mycobacteria	*Mycobacterium tuberculosis* M. bovis
	Bacterial Endospores	*Bacillus subtilis* *Clostridium sporogenes*
Most Resistant	Prions	

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. It is very important to evaluate each material being used and select the proper personal protective equipment (e.g., eye/face, gloves, respiratory) for each.

11.10.1 Common Disinfectants

11.10.1.1 Alcohols

Ethyl or isopropyl alcohol in concentration of 70% to 90% is good for general-use disinfection. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial and fungal spores. Concentrations above 90% are less effective. They are also flammable.

11.10.1.2 Phenol and Phenol Derivatives

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

11.10.1.3 Quaternary Ammonium Compounds (Quats)

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

11.10.1.4 Halogens (Chlorine and Iodine)

Chlorine-containing solutions have broad spectrum activity. Sodium hypochlorite (bleach) is the most common base for chlorine disinfectants. Common household bleach (5% – 10% sodium hypochlorite) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Use freshly prepared solutions for decontamination. Chlorine-containing disinfectants are inactivated by excess organic materials. If you need to disinfect material with a high organic load, increase concentration to a 1/5 dilution for 30-60 minutes. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. Iodine has similar properties to chlorine. Iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

11.11 Biohazard Waste Disposal

Per State of California regulations, Regulated Medical Waste includes:

- **Cultures and stocks of infectious agents and associated biologicals:** All discarded cultures and stocks of infectious agents and associated biotechnological by-product effluents, cultures of specimens from medical and pathological laboratories, cultures and stocks of infectious agents from research laboratories, wastes from the production of biologicals, and discarded live and attenuated vaccines, intended for human use.

- **Blood and blood products:** Discarded bulk human blood and blood products in free draining, liquid state; body fluids contaminated with visible blood; and materials saturated/dripping with blood.

- **Pathological waste:** Animal carcasses, tissues, body fluid soaked bedding of research animals.

- **Sharps:** Discarded medical articles that may cause puncture or cuts, including but not limited to all used and discarded hypodermic needles and syringes, Pasteur and serological pipettes, broken medical or bio-contaminated glassware, scalpel blades, disposable razors, and suture needles. This includes sharps that may not be biologically contaminated, such as a Pasteur pipette used with water.

- **Biotechnological by-product effluent:** Any discarded preparations made from genetically altered living organisms and their products.

11.11.1 General Labeling, Packaging and Disposal Procedures

Solid biohazardous waste is placed in red biohazard bags to be picked up and disposed of by a licensed medical waste contractor. The autoclaves are not used to decontaminate biohazardous waste.

Sharps are collected in leak and puncture resistant containers. The containers and their contents are decontaminated and destroyed by a licensed medical waste contractor.

Pathological (i.e., animal parts) waste is stored frozen until pick-up by a medical waste contractor.

Liquid biohazardous waste is disposed of by the following described methods:

- Liquid waste will be treated with a 10 – 20% solution of bleach for at least 30 minutes prior to drain disposal.

Biological Waste Segregation Table

Table 4. Biological Waste Segregation Table

Waste Type	Container	Disposal Method
Cultures	Red biohazard bag	Medical waste vendor
Human tissues	Red biohazard bag	Medical waste vendor
Sharps	Approved sharps container	Medical waste vendor
Liquid biological waste	Bleach treatment	Sanitary sewer
Animal tissue	Frozen pathological waste	Medical waste vendor

11.12 Transportation of Biological Materials Between Facilities

All biological materials shall be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release and

endangerment of the public and the environment. In addition, the transportation must comply with DOT regulations for ground transportation, and IATA requirements for transportation via air.

11.12.1 Transportation In-between Chapman University Buildings:
The transportation of all biological material shall follow the following specifications:

- Microbes, biological toxins, human and non-human primate materials, and recombinant or synthetic nucleic acid molecules must be packaged in a sealed, leakproof primary container which is securely positioned in a secondary leakproof and closable container. For transportation in-between buildings, a cooler or ice chest may be used and must contain a clearly visible biohazard label.

- A list of contents as well as emergency contact information (e.g., PI phone number) shall accompany the material (e.g., attached to the cooler in a plastic pouch);

- For the transportation of such materials on or off Chapman University campus property, contact EHS for guidance.

11.12.2 Transportation and Shipment Via Carrier Off Chapman University Property
- Transportation of biological material off of property of Chapman University, is regulated by national (DOT) and international (IATA) requirements;

- Only trained personnel can prepare and offer shipments for transportation. Contact the EHS Office for additional information;

- The shipment of diagnostic and clinical specimens, biological products, infectious agents and recombinant DNA molecules require:
  - Specific procedures for packing
  - Specific containers
  - Labeling
  - Documentation
  - Permits (may be required)

12.0 EMERGENCY PROCEDURES

Laboratory emergencies may result from a variety of factors, including serious injuries, fires and explosions, spills and exposures, and natural disasters. All laboratory employees should be familiar with and aware of the location of their laboratory's emergency response plans and safety manuals. Before beginning any laboratory task, know what to do in the event of an emergency situation. Identify the location of safety equipment, including first aid kits, eye washes, safety showers, fire extinguishers, fire alarm pull stations, and spill kits. Plan ahead and know the location of the closest fire alarms, exits, and telephones in your laboratory. The Chapman Emergency Procedure poster provides an overview of emergency response procedures and should be posted in each laboratory. If a copy is needed, please contact EHS.

12.1 Accidents and Incidents

PI and Laboratory Managers are responsible for ensuring that their employees receive appropriate medical attention in the event of an occupational injury or illness. All accidents and near misses must be reported to the supervisor and EHS. An injury, incident or safety concern can also be reported to EHS online https://www.chapman.edu/faculty-staff/risk-management/reporting.aspx. EHS will conduct an accident investigation and develop recommendations and corrective actions to prevent future accidents. At a minimum, each laboratory must have the following preparations in place:

- Fully stocked first aid kit
- Posting of emergency telephone numbers and locations of emergency treatment facilities

If an employee has a severe or life-threatening injury, call for emergency response at 911. Employees with minor injuries should be treated with first aid kits as appropriate and sent to the appropriate facility for further evaluation and treatment. Treatment can be obtained after normal business hours at designated medical centers and emergency rooms.

Serious occupational injuries, illnesses, and exposures to hazardous substances including potential pathogens must be reported to the supervisor and EHS within 8 hours. EHS will report the event to Cal/OSHA and/or NIH, investigate the accident, and complete exposure monitoring, if necessary. As soon as Faculty/ Laboratory Supervisors are aware of a potentially serious incident, they must contact EHS.

12.2 Fire-Related Emergencies

During a fire emergency, lab staff should prioritize life safety. Cultures and animals may be put away if time allows; if not, walk to the nearest exit. If you encounter a fire, or a fire-related emergency (e.g., abnormal heating, smoke, burning odor), immediately follow these instructions:

1. Pull the fire alarm pull station and call Public Safety
2. Evacuate and isolate the area
    a. Use portable fire extinguishers to facilitate evacuation and/or control a small fire (i.e., size of a small trash can), if safe to do so and if properly trained
    b. If possible, shut off equipment before leaving
    c. Close doors and/or fume hood sash
3. Remain safely outside the affected area to provide details to emergency responders
4. Evacuate the building when the alarm sounds. It is against state law to remain in the building when the alarm is sounding. If the alarm sounds due to a false alarm or drill, you will be allowed to re-enter the building as soon as the Fire Department determines that it is safe to do so. Do not go back in the building until the alarm stops and you are cleared to reenter.
5. If your clothing catches on fire, go to the nearest emergency shower immediately. If a shower is not available, then stop, drop, and roll. A fire extinguisher may be used to extinguish a fire on someone's person. Report any burn injuries to the supervisor immediately and seek medical treatment. Report to the EHS within 8 hours every time a fire extinguisher is discharged.

12.3 Power outage

In the event of a power outage, put away cultures and animals. Remove PPE and exit the lab normally. Emergency lighting within the buildings should provide adequate visibility to exit the building.

12.4 Exposure to potential pathogens

In the event of contact with blood or other potentially infectious material through non-intact skin, such as a rash, acne, or hangnail; through the eyes, nose, or mouth; or by events that break skin or mucous membrane barrier, such as a needlestick, cut, abrasion, or human bite, follow these procedures:

- Remove contaminated items and place them in a biohazard bag or receptacle.
- Minimize the exposure by immediately flooding the exposed area with water, then thoroughly wash the affected skin with soap and water.
- Flush any splashes to your nose and mouth with water for at least 15 min.
- Irrigate your eyes with water or saline if they were exposed.
- Report the incident to your PI or supervisor so that a record of the exposure can be documented, a medical evaluation can be made available to you if required, and your employer can look for ways to prevent a similar incident from happening again.
- Be sure to seek medical attention as soon as possible after the incident.)

13.0 REFERENCES

Federal Biosafety Guidance

CDC and NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition. Revised June 2020.
https://www.cdc.gov/labs/bmbl/index.html
https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

NIH Office of Science Policy. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. April 2024.
https://osp.od.nih.gov/biotechnology/nih-guidelines/
https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

California Regulations

California Code of Regulations Title 8 Section 5193. Bloodborne Pathogens Standard.
https://www.dir.ca.gov/title8/5193.html

California Code of Regulations Title 8 Section 5199. Aerosol Transmissible Diseases Standard.
https://www.dir.ca.gov/title8/5199.html

California Medical Waste Management Act (Health and Safety Code Sections 117600-118360).
https://leginfo.legislature.ca.gov

Biological Agents and Select Agents

Federal Select Agent Program.
https://www.selectagents.gov

HHS 42 CFR Part 73 Possession, Use, and Transfer of Select Agents and Toxins.
https://www.ecfr.gov

USDA 7 CFR Part 331 and 9 CFR Part 121 Agricultural Select Agents and Toxins.
https://www.ecfr.gov

Import, Export, and Shipping

U.S. DOT Hazardous Materials Regulations, 49 CFR Parts 171-180.
https://www.ecfr.gov/current/title-49/subtitle-B/chapter-I

International Air Transport Association (IATA) Dangerous Goods Regulations.
https://www.iata.org

CDC Import Permit Program.
https://www.cdc.gov/importation

USDA APHIS Permits for Movement of Organisms and Biological Materials.

https://www.aphis.usda.gov

Occupational Health and Laboratory Safety

OSHA Occupational Exposure to Bloodborne Pathogens, 29 CFR 1910.1030.
https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030

National Research Council. Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards.
https://nap.nationalacademies.org

Biosafety Cabinet Guidance

CDC Biosafety Cabinet Resources.
https://www.cdc.gov/labs

NSF/ANSI 49 Biosafety Cabinetry Standard.
https://www.nsf.org

Pathogen Information Resources

Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment Database.
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

Professional Organizations

ABSA International.
https://absa.org

Chapman University References

Biological Safety Program

Bloodborne Pathogen Exposure Control Plan

https://www.chapman.edu/faculty-staff/environmental/biological.aspx

Chemical Hygiene Plan

Hazardous Waste Management Program

https://www.chapman.edu/faculty-staff/environmental/research-and-laboratory-safety.aspx

Incident Reporting System
https://www.chapman.edu/faculty-staff/risk-management/reporting.aspx