BIOSAFETY MANUAL

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I. Introduction & Purpose

This manual is designed as a reference and guide for the protection of employees, students, and the community from potential health hazards arising from the use of infectious agents or recombinant or synthetic nucleic acid molecules in various laboratory settings.

The biosafety manual for the College of Natural Science and Mathematics (NSM) at California State University, Sacramento (CSUS) has been adopted to achieve the following goals:

- Protect personnel from exposure to infectious agents
- Prevent environmental contamination
- Comply with applicable federal, state, and local requirements

The biosafety manual provides college-wide guidelines and recommendations for individual laboratory safety policies and procedures for the use and manipulation of biohazards. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used and is dependent on the combined efforts of laboratory supervisors (Principal Investigator, Course Coordinator, or Instructor of Record), instructional support technicians, employees, volunteers, and students. In general, the handling and manipulation of biological agents and toxins, as well as recombinant or synthetic nucleic acid molecules, requires the use of various precautionary measures depending on the material involved. This manual will provide guidance 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.

II. General Definitions & Acronyms

- **Aerosols** - Colloids of liquid or solid particles less than 10 microns in diameter suspended in air.
- **Autoclave** - A device designed to sterilize equipment or biological waste using heat, steam, and pressure within a chamber.
- **Biological Agent** – Living cells, microbes, or viruses, infectious agents, and recombinant DNA molecules.
- **Biohazardous Agent** – Capable of causing disease in humans, animals, or plants. These include, but are not limited to: viruses, microorganisms, parasites, recombinant products, cultured human or animal cells, and subviral agents. The term "agent" includes the agent, products of infectious agents, or the components of infectious agents presenting a risk of illness or injury.
- **Biohazardous Materials** - Any materials that would harbor biohazardous agents such as human blood, body fluids, or tissues that may be contaminated with biohazardous agents.
- **Biological Barrier** - An impediment (naturally occurring or introduced) to the infectivity and/or survival of a biohazardous agent.
- **Biological Safety Cabinet** - A device enclosed on three sides; the top and bottom are designed to draw air inward by means of mechanical ventilation. Exhaust air is filtered and/or ducted to the outside. Used for the manipulation of biological and biohazardous agents.
- **Biological Toxins** – Any poisonous substances produced by microorganisms, animals, or plants. This includes metabolites from living organisms, degraded products from nonliving organisms, and materials rendered toxic by the metabolic activity of microorganisms. Since toxins cannot replicate, they are not considered infectious. Many biological toxins are highly toxic in very small quantities, therefore must be managed as hazardous. Examples include bacterial toxins like botulinum toxin, tetanus toxin, staphylococcal enterotoxins, diphtheria toxin, and pertussis toxin; animal toxins like tetrodotoxin, conotoxin, snake venom toxins and ciguatoxin; and plant or fungal toxins like ricin toxin, abrin, and trichothecene mycotoxins.
- **Biosafety Level** - Laboratory practices, techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazards posed by the particular biohazardous material. The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) define four levels of biosafety and four levels of animal biosafety. Biosafety levels of specific agents are listed in the Biosafety Levels for Infectious Agents (Appendix 1).
- **Bloodborne Pathogens** - Pathogenic microorganisms that are present in human and other primate blood and can cause diseases in humans. These pathogens include, but are not limited to, Hepatitis B Virus (HBV,) Hepatitis C Virus (HCV,) and Human Immunodeficiency Virus (HIV.)
- **CDC** - Centers for Disease Control and Prevention.
- **Containment** - The confinement of a biohazardous agent that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people

and/or the environment. Methods used to achieve this include physical and biological barriers and inactivation using physical or chemical means.

- **Decontamination** - The removal or neutralization of toxic agents or the use of physical or chemical means to remove, inactivate, or destroy living organisms on a surface or item so that the organisms are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.
- **Disinfection** - A process by which viable biohazardous agents are reduced to a level unlikely to produce disease in healthy humans, plants, or animals.
- **Engineering Controls** - Controls (e.g., sharps containers, self-sheathing needles) that isolate or remove pathogens from the workplace.
- **EH&S** - Environmental Health & Safety Department.
- **Exposure Incident** - Contact with blood or OPIM that results from the performance of an employee's duties.
- **High Efficiency Particulate Air (HEPA) Filter** - A disposable, extended, pleated-medium, dry-type filter with (1) a rigid casing enclosing the full depth of the pleats, (2) a minimum particulate removal of 99.97 percent for thermally generated monodisperse dioctyl phthalate (DOP) smoke particles with a diameter of 0.3 um, and (3) a maximum pressure drop of 1.0 in. Hg when clean and operated at rated airflow capacity.
- **Institutional Biosafety Committee (IBC)** - IBCs were established under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. The role of the IBC is to provide local review and oversight of experimentation (in both research and teaching settings) utilizing recombinant or synthetic nucleic acid molecules, biological materials (e.g., infectious agents), and potentially hazardous agents.
- **Inactivation** - Any process that destroys the ability of a specific biohazardous agent to self-replicate.
- **Laboratory-Acquired Infection (LAI)** - All infections acquired through laboratories or laboratory-related activities, whether they are symptomatic or asymptomatic in nature.
- **Laminar Airflow** - Unidirectional airflow through the work area often referred to as (1) turbulence-free airflow, (2) steady unidirectional microturbulence flow, or (3) mass airflow.
- **Medical Waste Management Act (MWMA)** - California Health and Safety Code, Division 20, Chapter 6.1, Commencing with Section 25015.
- **Mixed Waste** - Waste that contains more than one type of hazardous constituent. For example, waste that is contaminated with biohazardous, radioactive, and chemical substances is considered mixed waste.
- **NIH** - National Institutes of Health.
- **NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)** - *NIH Guidelines* detail safety practices and containment procedures for basic

and clinical research involving recombinant or synthetic nucleic acid molecules, including the creation and use of organisms and viruses containing recombinant or synthetic nucleic acid molecules.

- **Occupational Exposure** - Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or OPIM that may result from the performance of an employee's duties.
- **OPIM** - Other Potentially Infectious Materials. OPIM include, but are not limited to: mixed body fluids where it is difficult to differentiate between composite body fluids, amniotic fluid, any bodily fluid visibly contaminated with blood, unfixed human tissues or organs, blood/organ/tissue from experimental animals infected with bloodborne pathogens, cell/tissue/organ cultures containing bloodborne pathogens, cerebrospinal fluid, culture media or other solutions containing bloodborne pathogens, semen, vaginal secretions, fecal material, peritoneal fluid, pleural fluid, and saliva in dental procedures.
- **OSHA** - Occupational Safety and Health Administrations.
- **Parenteral** - Piercing mucous membrane or the skin barrier through events such as needlesticks, human bites, cuts, and abrasions.
- **Pathogen** - Any biohazardous agent that is capable of producing disease in healthy humans, plants, or animals.
- **Personal Protective Equipment (PPE)** - Specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) not intended to function as protection against a hazard is not considered to be personal protective equipment.
- **Personal Protection** - Techniques or devices designed to eliminate or significantly reduce employee risk.
- **Physical Barrier** - Any equipment, facilities, or devices designed to achieve containment or exclusion.
- **Recombinant DNA (rDNA)** - Either (1) molecules constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (2) DNA molecules that result from the replication of those described above.
- **Sharps** - Instruments, tools, or items that have rigid and acute edges, protuberances, or corners capable of cutting, piercing, ripping, or puncturing. These include syringes, blades, and broken glass. Items that have the potential for shattering or breaking are also considered sharps.
- **Standard Operating Procedure (SOP)** - Set of step-by-step instructions compiled by an organization to help workers carry out routine operations. SOPs aim to achieve efficiency, quality output, and uniformity of performance, while maximizing safety and reducing miscommunication and failure to comply with regulations.

- **Standard Precautions** - Infection control guidelines promoted by the Centers for Disease Control and Prevention to mitigate the threat of employee exposure to human blood and/or certain body fluids.
- **Sterilize** - The use of a physical or chemical procedure to destroy all microbial life including highly resistant bacterial endospores.

III. What are Biohazards?

A ***biohazard*** is a biological agent or material which is potentially hazardous to humans, animals, and/or plants. For this manual, the definition of a biohazard includes infectious or etiologic (disease causing) agents, other potentially infectious materials (OPIM), certain toxins of biological origin, some recombinant or synthetic nucleic acids, and other hazardous biological materials.

- Biohazardous agents may include but are not limited to: *certain bacteria, fungi, viruses, rickettsiae, chlamydiae, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents these cells may contain, including viroids, prions and other infectious agents as outlined in laws, regulations and guidelines.*

Biological hazards can be found in the various teaching, research, and support environments in the College of Natural Science and Mathematics (NSM). Current activities in NSM only include agents from Risk Group 1 (RG-1) or Risk Group 2 (RG-2), used in Biosafety Level 1 (BSL-1) or Biosafety Level 2 (BSL-2) laboratories.

1. Infectious Agents

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

Table 1. Basis for the Classification of Biohazardous Agents by Risk Group

Risk Group	Risk to the Individual and the Community
Risk Group 1 (RG-1)	Agents that are not associated with disease in healthy adult humans.
Risk Group 2 (RG-2)	Agents that are associated with human disease that are rarely serious and for which preventive or therapeutic interventions are often available.

Risk Group 3 (RG-3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk, but low community risk).
Risk Group 4 (RG-4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual and community risk).

2. Other Potentially Infectious Materials (OPIM)

- The Occupational Safety and Health Administration (OSHA) regulates occupational exposure to bloodborne pathogens and mandates a combination of engineering and work practice controls, annual training, Hepatitis B vaccination, and other provisions to help control the health risk to employees resulting from occupational exposure to human blood and OPIM that may contain these or other specified agents.
- Human or non-human primate source materials are considered biohazards and shall be handled consistent with Biosafety Level 2 (BSL-2) practices and procedures. All specimens of human or non-human primate blood, blood products, body fluids, unfixed tissues and organs, and human or other primate cells lines (established or primary) are to be treated as if they are known or suspected to be infectious.
- **Exception:** materials fixed prior to receipt

3. Biological Toxins that are Considered Biohazards

- Any biological toxin with an LD50 of 100 microgram/kilogram body weight or less
- Any newly discovered biological toxin for which the LD50 has not been determined
- Any biological toxin covered under the *NIH Guidelines* (any experiment involving the cloning of biological toxin molecules with an LD50 of less than 100 nanograms per kilogram body weight)
- A biological toxin is any poisonous substances produced by microorganisms, animals, or plants. This includes metabolites from living organisms, degraded products from nonliving organisms, and materials rendered toxic by the metabolic activity of microorganisms.
- Examples include bacterial toxins like botulinum toxin, tetanus toxin, staphylococcal enterotoxins, diphtheria toxin, and pertussis toxin; animal toxins like tetrodotoxin, conotoxin, snake venom toxins and ciguatoxin; and plant or fungal toxins like ricin toxin, abrin, and trichothecene mycotoxins.

4. Recombinant or Synthetic Nucleic Acids

A. Recombinant and Synthetic Nucleic Acids

According to the *NIH Guidelines*, are defined as:

i. Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids
ii. Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids
iii. Molecules that result from the replication of those described in (i) or (ii) above

- Experiments involving the generation of recombinant or synthetic nucleic acids, including the purchase, creation or use of any transgenic material, may require review and approval by the IBC.
- **Exceptions:** *in vitro* use of nucleic acids (i.e., PCR, DNA sequencing) that does not involve the cloning and propagation of recombinant or synthetic DNA in cells

B. Transgenic Animals

- Creation or use of transgenic animals (vertebrate or invertebrate) involves the cloning and propagation of recombinant or synthetic DNA in cells, therefore is considered a biohazard.

C. Transgenic Plants

- Experiments to genetically engineer plants by recombinant DNA methods involves the cloning and propagation of recombinant or synthetic DNA in cells, therefore is considered a biohazard. The *NIH Guidelines* provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineering plants.

5. Other Hazardous Biological Materials

- Animals or animal-derived products that harbor zoonotic agents (e.g., wild trap animals, fecal samples from wild rodents)
- Environmental samples collected from areas that may contain infectious agents
- Plants or plant products that are non-indigenous or noxious weeds
- Large scale cultures of over 10 liters in one vessel

IV. Biological Risk Assessment

The assessment of risk is an essential element of safety in the laboratory. While hazards are defined as substances or situations capable of causing adverse effects to health or safety, risks occur when people interact with hazards. Since it is impossible to eliminate all risk, unless the hazard is eliminated, the risk assessment evaluates a hazard's known risks and reduces risk to an acceptable level to protect personnel, the community, and the environment. The biological risk assessment process is used to:

- Identify the hazardous characteristics of an infectious or potentially infectious agent or material, if known.
- Identify the laboratory procedures/activities that can result in a person's exposure to an agent.
- Determine the likelihood that such exposure will cause a Laboratory Associated Infection (LAI).
- Determine the probable consequences of such an infection.

The information identified by risk assessment will help determine which Biosafety Level, good microbiological practices, safety equipment, training, waste disposal, and facility safeguards can help prevent LAIs. As all possible adverse incidents can't be predicted, judgments and decisions sometimes need to be based on incomplete information. When there is insufficient information to make a clear determination of risk, additional safeguards are generally preferred.

Outlined below are a series of general steps to follow when conducting a biological risk assessment:

1. **Step One: Identify hazardous characteristics of the agent and perform an assessment of the inherent risk.** Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible host, severity of disease, and the availability of preventive measures and effective treatments. Also consider possible routes of transmission of infection in the laboratory, infectious dose, stability in the environment, host range, whether the agent is indigenous or exotic to the local environment, and the genetic characteristics of the agent.

    - **Genetically Modified Agents:** The *NIH Guidelines* are the key reference in assessing risk and establishing an appropriate Biosafety Level for work involving recombinant DNA molecules. It is particularly important to address the possibility that the genetic modification could increase or decrease an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments.

    - **Cell Cultures:** Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. In addition, human and animal cell lines that are not

well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory.

- Once the inherent risk associated with the agent is considered, address the possibility of transmission of the agent. The most likely routes of transmission in the laboratory are:
    1. Direct skin, eye or mucosal membrane exposure to an agent;
    2. Parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors;
    3. Ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and
    4. Inhalation of infectious aerosols.

- Often, there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a specimen may not be known. In this case, assume the specimen contains an unknown agent that correlates with a minimum of BSL-2 containment, unless additional information suggests the presence of an agent of higher risk.

2. **Step Two: Identify laboratory procedure hazards.** The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

    - **Aerosols and Droplets** Procedures that impart energy to a microbial suspension will produce aerosols. Equipment such as pipettes, blenders, centrifuges, sonicators, and vortex mixers are potential sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods. Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of exposure. Procedures and equipment that generate respirable size particles also generate larger size droplets that settle out of the air rapidly, contaminating hands, work surfaces, and possibly the mucous membranes of the persons performing the procedure.

    - **Personal Protective Equipment (PPE) and Safety Equipment Hazards** There may be hazards that require specialized PPE in addition to safety glasses, laboratory coats, and gloves. Safety equipment such as biological safety cabinets (BSCs), centrifuge safety cups, and sealed rotors

are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets.

- **Facility Control Hazards** Facility safeguards help prevent the accidental release of an agent from the laboratory. For example, one facility safeguard is directional airflow, which helps to prevent aerosol transmission from a laboratory into other areas of the building. A biological safety professional, building and facilities staff, and the Institutional Biosafety Committee, should help assess the facility's capability to provide appropriate protection for the planned work and recommend changes as necessary.

3. **Step Three: Determine the appropriate Biosafety Level and select additional precautions indicated by the risk assessment.** Biosafety Levels are outlined in Table 2. It is important to note that the biosafety plan may be influenced by federal regulations and guidelines. For example, the National Science Foundation (NSF) defines standard terms and conditions for federal research grants. It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Consultation with an occupational health care provider knowledgeable in infectious diseases is advisable in these circumstances.

4. **Step Four: Before implementation, review the risk assessment with a biosafety professional, subject matter expert, and the IBC or equivalent resource.** This review is strongly recommended and may be required by regulatory or funding agencies. Examples of who to contact for review include: University level biosafety officer, College level safety officer, senior researchers or staff familiar with the subject, members of the IBC.

5. **Step Five: Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.** The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. Laboratory workers must acquire technical proficiency and develop good habits in the use of microbiological practices and safety equipment required for the safe handling of the agent. Staff at all skill levels need to know how to identify hazards in the laboratory and how to obtain assistance in protecting themselves and others in the laboratory.

   To reduce the risks associated with hazardous agent, regular evaluation is recommended with respect to a worker's:
   - Training
   - Knowledge of the agent and procedure hazards
   - Experience in handling infectious agents

- Proficiency in following good microbiological practices
- Correct use of safety equipment
- Consistent use of standard operating procedures (SOPs) for specific laboratory activities
- Ability to respond to emergencies
- Willingness to accept responsibility for protecting one's self and others
- Concern for the health of coworkers
- Good habits, caution, and attentiveness

6. **Step Six: Revisit regularly and verify risk management strategies and determine if changes are necessary.** Continue the risk management cycle, and adjust and adapt as the need arises. This includes a regular update of biosafety manuals and SOPs when changes in procedures or equipment occur.

V. Biosafety Principles & Practices

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

1. **Primary Containment**
    - Protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment such as a biological safety cabinet (BSC).
2. **Secondary Containment**
    - Protection of the environment external to the laboratory from exposure to infectious materials through a combination of facility design and operational practices.
    - Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment.
3. **Biosafety Levels**
    - Currently four Biosafety Levels (1-4) define the level of containment necessary to protect personnel and the environment. Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Level 3 and 4 (BSL-3 and BSL-4) require special containment laboratories or facilities, which are not available at CSUS (please see Appendix 3, Table 5 for summary of BSL-3, BSL-4, and ABSL-3). Teaching, research and diagnostic laboratories at CSUS are conducted at BSL-1 and BSL-2.

Table 2. Summary of Biosafety Levels for Infectious Agents (BSL-1 & BSL-2) *

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

Biosafety Level 2 (BSL-2)	
Agents:	Associated with human disease, hazard exposure = auto-inoculation, ingestion, mucous membrane exposure
Practices:	BSL-1 practice **plus**: Limited access; biohazard warning signs; "Sharps" precautions; biosafety manual defining any needed training; waste decontamination or medical surveillance policies
Safety Equipment: (Primary Barriers)	Biological Safety Cabinet (BSC) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; Personal Protective Equipment (PPE): Laboratory coat, gloves, eye/face protection, closed-toed shoes
Facilities: (Secondary Barriers)	BSL-1 facility **plus**: Controlled access; waste to be classified as medical waste and disposed of following University procedures; equipment decontamination before removal from laboratory

Table 3. Summary of Biosafety Levels for Infectious Agents with Vertebrate Animals Use *

Animal Biosafety Level 1 (ABSL-1)	
Agents:	Not known to cause disease in healthy adults
Practices:	Standard animal care and management practices, including medical surveillance
Safety Equipment: (Primary Barriers)	As required for normal care of each species.
Facilities: (Secondary Barriers)	Standard animal facility; non-recirculation of exhaust air; directional air flow recommended
Animal Biosafety Level 2 (ABSL-2)	
Agents:	Associated with human disease, hazard exposure = percutaneous exposure, ingestion, mucous membrane exposure
Practices:	ABSL-1 practice **plus**: Limited access; biohazard warning signs; "Sharps" precautions; biosafety manual defining any needed training; waste decontamination; animal cage decontamination prior to washing
Safety Equipment: (Primary Barriers)	ABSL-1 equipment **plus**: Biological Safety Cabinet (BSC) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; containment equipment appropriate for animal species; Personal Protective Equipment (PPE): Laboratory coat, gloves, eye/face

	protection, closed-toed shoes, and respiratory protection (as needed)
Facilities: (Secondary Barriers)	ABSL-1 facility **plus**: Controlled access; autoclave available; handwashing sink in animal room; waste to be classified as medical waste and disposed of following University procedures; equipment decontamination before removal from laboratory

** These tables were adapted from the 6th Edition of the "Biosafety in Microbiological and Biomedical Laboratories" Handbook. (See Appendix 1 for recommended Biosafety Levels for specific infectious agents).*

4. Containment Practices and Procedures

Following are practices and common-sense principles that protect personnel, the experiment, and the environment.

1. Standard Microbiological Practices

- Access to the laboratory may be limited or restricted at the discretion of the laboratory supervisor when experiments or work with cultures and specimens are in progress.
- Decontaminate work surfaces after use, after any spill of viable materials, and at least once per day.
- Eating, drinking, smoking, applying cosmetics, handling contact lenses, and storing food are prohibited in work areas. Food must be stored in cabinets or refrigerators solely designated for this purpose and should be located outside the work area.
- Use mechanical pipetting devices; mouth pipetting is prohibited.
- Restrict the use of needles and syringes to those procedures for which there are no alternatives; use needles, syringes, and other "sharps" carefully to avoid self-inoculation, and dispose of "sharps" in leak- and puncture-resistant containers.
- Policies for the safe handling of sharps are instituted.
- Wash hands after handling cultures or animals, after removing gloves, and before leaving the room.
- Carefully perform all procedures to minimize the creation of aerosols and splashes.
- Protective eyewear should be worn for activities and procedures in which splashes are anticipated.
- Laboratory coats or gowns are recommended.
- Wear lab coat, gloves, and safety glasses to prevent contamination from the infectious material, and remove protective equipment prior to leaving the work area.

2. Special Practices for Biosafety Level 2 (BSL-2)

- Biological Safety Cabinets (BSCs) or other appropriate combinations of personal protective equipment and physical containment devices (centrifuge safety cups, sealed centrifuge rotors, containment caging for animas) should be used for:
    - procedures with a high potential for created infectious aerosols
    - procedures using high concentrations or large volumes of infectious agents
- Handle all liquid and solid waste as though infectious.
- Decontaminate work surfaces on completion of work or at the end of the day and after any spill or splash of viable material; use disinfectants that are effective against the agents of concern.
- Dispose of all biohazardous waste in accordance with applicable regulations.
- Wash hands after handling any biohazardous material and before leaving the laboratory.
- Take special care to avoid skin contamination with infectious material; gloves should be worn when skin contact with infectious material is unavoidable.
- Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

3. Safety Equipment

The risk of exposure of laboratory personnel can be minimized by the use of carefully selected safety equipment. This safety equipment should effectively isolate the worker from the toxic or infectious material being processed.

- Biological Safety Cabinets (BSCs) are used to prevent the escape of aerosols and droplets and to protect materials from airborne contamination. (See Appendix 4 for additional information about BSCs).
- Personal Protective Equipment (PPE) such as long pants and closed-toed shoes are required before entering any CSUS laboratory. Laboratory coat, gloves, and safety glasses are required for Biosafety Level 2 (BSL-2). Specialized PPE may be required for specific hazards. For example, a procedure that presents a splash hazard may require the use of a mask and face shield to provide adequate protection.

4. Biohazard Signs and Labels

- For laboratories and animal rooms requiring BSL-2 precautions, signs indicating that biohazardous agents are used within the room must be posted at the entrances.
    - The sign must include the universal biohazard symbol, specific entry requirements, and the name and telephone number of the PI and/or other responsible persons.
- Labels including the universal biohazard symbol and the word "Biohazard" must be placed on equipment such as refrigerators, freezers, incubators, shipping containers, primary and secondary agent containers, and any surface that may be reasonably anticipated to have surface contamination from biohazardous agents.

5. Biological Waste

For biological waste information, refer to the CSUS Medical Waste Management Plan.

- All medical waste except sharps must be placed in red bags that are labeled with the universal biohazard symbol and the words "Biohazard" or "Biohazardous Waste."
- Include the Department name, building name, and room number on the bags.
- Medical waste sharps containers must also be labeled with the universal biohazard symbol, the words "Biohazard" or "Biohazardous Waste," and the location information.
- Secondary containers for medical waste must be labeled on all sides and on the lid with the universal biohazard symbol and the words "Biohazard" or "Biohazardous Waste."

6. Emergency Procedures

- All laboratories working with biohazards should establish written emergency procedures based on the biohazardous agents used and any other hazards that may be present. The following items should be included:
    - First, attend to injured personnel. Call 911 on any campus phone or (916) 278-6000 from a cell phone. Inform responders of biohazards that may be a threat.
    - For spills in BSL-2 laboratories, evacuate the room and close the doors.
    - After evacuating the area, wait to assist emergency responders.
- See Appendix 5 for detailed Biohazardous Spill Clean-up Protocols.
- Any accidents, exposure, or biohazard spills must be reported. (See "Incident Reporting" in next section).

VI. Requirements & Surveillance

1. Experiments Requiring IBC Review / Approval

(Sac State does not currently have an Institutional Biosafety Committee (IBC), therefore does not have an institutional policy or procedure for review and approval of experiments involving biohazards. The following are suggested guidelines for what experiments may require IBC review and approval in the future.)

Institutional Biosafety Committee (IBC) review and/or approval may be required for any experiment in a research, teaching, or support environment that includes one or more of the following:

- Recombinant and synthetic nucleic acid molecules and the cells, organisms and viruses containing such molecules. This includes the purchase, creation, or use of any transgenic material. Recombinant and synthetic nucleic acid molecules are defined as:
    i. Molecules that are a) constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids.
    ii. Nucleic acid molecules that are chemically or by other means synthesized or amplified including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids.
    iii. Molecules that result from the replication of those described in (i) or (ii) above.
        - **Exception**: *in vitro* use of nucleic acids (i.e., PCR, DNA sequencing) that does not involve the cloning and propagation of recombinant DNA in cells
- Biological microorganisms, regardless of Risk Group (e.g., bacteria, viruses, fungi, protozoa, prions, cell lines)
    - Includes any human, animal, and/or plant pathogen
    - Includes acquisition and storage of new biological agents, or for procedures that significantly increases the risk hazard for an existing biological agent.
- Human or non-human primate source materials
    - Includes cell lines (established or primary), tissues, blood, blood products, body fluids
    - **Exception**: materials that were fixed prior to receipt
- Biological Toxins (possession, use, and/or transfer)
    - With an LD50 of 100 microgram/kilogram body weight or less
    - Any newly discovered toxin for which the LD50 has not been determined
    - Any toxin covered under the *NIH Guidelines* (any experiment involving the cloning of toxin molecules with an LD50 of less than 100 nanograms per kilogram body weight)
- Animals or animal-derived products that harbor zoonotic agents (e.g., wild trap animals, fecal samples from wild rodents)
- Plants or plant products that are non-indigenous or noxious weeds

- Large scale cultures of over 10 liters in one vessel
- Environmental samples collected from areas that may contain infectious agents

2. Inspections

Laboratory inspections will be performed periodically by EH&S. These inspections are designed to verify that all laws and regulations outlined in this manual and by state and federal organizations are followed. This includes the use of adequate facilities and proper maintenance of these facilities. Additionally, interviews of laboratory personnel may be conducted to ensure they are aware of proper safety and emergency protocols and are informed about the properties of the organisms with which they are working. Training records and other documentation may be reviewed at this time.

3. Training

Training is required for all employees, including students and volunteers, working in labs or animal rooms where biological agents are used. Training requirements should be followed in accordance with the CSUS Injury Illness Prevention Program and Bloodborne Pathogen Exposure Control Plan. The PI, laboratory director, or animal facility director (whoever has supervisory authority over personnel) is responsible for ensuring that adequate instruction is provided to all personnel who will have contact, or will be involved with, biological agents. This includes training for specific tasks that employees will perform. All training must be documented and the signed documents must be kept in the lab or with departmental records. Documentation shall include:

1. A description of the training
2. Date and location of the training
3. Name and title of the trainer; names and job titles of all individuals attending the training
4. Signatures of the trainer and trainees

4. Occupational Health and Medical Surveillance

- Vaccinations – Using certain biohazardous agents may require vaccinations for personnel. The EH&S Medical Monitoring Program may recommend vaccination for personnel exposed, or potentially exposed, to certain biohazardous agents as a result of their participation in a project.
- Medical Evaluations – After an exposure incident, the affected individual must receive a medical evaluation from the Student Health Center or CSUS's emergency medical provider. This includes collection of blood specimens for future analysis of personnel exposed, or potentially exposed, to certain biological agents.
- Cost for Medical Surveillance – The costs for medical surveillance will be borne by the appropriate departmental, research, or instructional budget. Medical surveillance costs for employees and students should be planned before conducting the research project.
- Health Service of Individuals Having Animal Contact

- Definition – The phrase "individuals having animal contact" refers to employees and students who, in the course of their employment, research, or education, have substantial contact with research animals used for biohazardous agent studies (e.g., animal caretakers, animal technicians, veterinarians) and where such contact may pose a threat to humans or animals.
- Responsibility – It is the responsibility of the immediate supervisor to establish whether the animal contact is substantial or poses a possible health threat. If the animals are live vertebrates, they would be subject to Institutional Animal Care and Use committee (IACUC) protocols and procedures. The supervisors of individuals having animal contact with notify campus EH&S.
- Refer to the CSUS Bloodborne Pathogen Exposure Control Plan for additional details.

5. Incident Reporting

- All injuries/illnesses/near misses should be reported to the PI or lab supervisor immediately. Please use common sense and reasonableness in evaluation of a near miss and/or consult with EH&S.
- Reporting procedures should be followed in accordance with the CSUS Injury Illness Prevention Program and Bloodborne Pathogen Exposure Control Plan.
- The PI or lab supervisor will work with the injured/ill or reporting person to complete the CSUS "Report of Incident or Accident" form.
- Forward the completed form to the Workers' Compensation Manager.

VII. Campus Policy

1. Responsibilities

- Environmental Health and Safety Department (EH&S)
    - Provide consultation/training to departments according to their specific needs
    - Provide information on the handling and disposal of biohazards
    - Identify and classify biological agents utilized at the University
    - Recommend personal protective equipment and engineering controls
    - Respond to emergency situations
- Department Chair
    - Assure the health and safety of employees, visitors, and students in CSUS facilities under department control
    - Ensure departmental compliance with applicable laws, regulations, and guidelines covering the use of biological agents in research
- Laboratory Supervisors (Principal Investigator (PI), Course Coordinator, or Instructor of Record)
    - Ensure that proper biological safety procedures are implemented and adhered to
    - Establish emergency procedures specific to the biohazards present
    - Clearly label all biohazards and biohazard work areas
    - Ensure all employees and students under their direction are trained with respect to biological safety and laboratory procedures
    - Document all training
- Instructional Support Technicians
    - Collaborate with Department Chair and Laboratory Supervisors on responsibilities consistent with specific job description (some of these responsibilities may be shared with others at a different category level)
    - Clearly label all biohazards and biohazard work areas
    - Ensure all employees and students under their direction are trained with respect to biological safety and laboratory procedures
    - Document all training
- Teaching Assistants, Laboratory Assistants, Students
    - Follow all established laboratory policies and procedures
    - Notify instructor/supervisor of accidents, violations, or unsafe conditions
    - Participate in all required biological safety training
    - Learn and use good standard microbiological practices
    - Correctly use safety equipment

2. Regulations, Guidelines, and Recommended Standards

- General
    - California Health and Safety Code Sections 117600-118360
    - CDC *Guidelines for Biosafety in Microbiological and Biomedical Laboratories, 6th edition*
    - *NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules*
    - Guidelines from the National Cancer Institute for work with oncogenic viruses
- Bloodborne Pathogens
    - Cal-OSHA, Title 8 of the California Code of Regulations (CCR) Section 5193, Bloodborne Pathogens
    - CSUS Bloodborne Pathogen Exposure Control Plan
- Medical Waste Management Act
    - Medical Waste Management Act (MWMA), California Health and Safety Code Division 20, Chapter 6.1 regulates the handling, storage, treatment and disposal of medical waste
    - State Department of Health Services (DHS) must permit medical waste treatment facilities, including autoclaves
- Biological Safety Cabinets
    - The certification, use, and maintenance of Class II biological safety cabinets is described in the NSF International Standard 49 (NSF Standard 49).
    - The use and maintenance of biological safety cabinets is regulated by Cal-OSHA in Title 8, CCR, Section 5154.2, Ventilation Requirements for Biosafety Cabinets.
- Biosafety Practices
    - Based on the CDC *Guidelines for Biosafety in Microbiological and Biomedical Laboratories, 6th edition*
- Biosafety Training
    - Mandated by Title 8, CCR, Section 3203, Injury and Illness Prevention Program (IIPP) and Title 8, CCR, Section 5193, Bloodborne Pathogens
- Recombinant DNA
    - *NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules*

VIII. Acknowledgements and References

We would like to thank and acknowledge the EH&S Departments and various College and University Dean's Offices for helpful documents and discussions about the Biosafety Programs at their Universities. This document is not an original work. Multiple provided documents were assembled together and modified to create this Biosafety Manual.

- California State University, Fullerton
- California State University, Channel Islands
- California State University, Monterey Bay

- California State University, Northridge
- Humboldt State University
- San Diego State University
- Carnegie Mellon University

IX. Appendices

Appendix 1: Biosafety Levels for Infectious Agents

The tables below are not an exhaustive list; if you work with an agent not listed, please perform a risk assessment and consult with the EH&S Department to determine the appropriate Biosafety Level.

Table 4. Biosafety Levels for Infectious Agents

Bacterial Agent	BSL	ABSL	Comments
Acinetobacter calcoaceticus	2	2	
Actinobacillus sp.	2	2	
Actinomyces sp.	2	2	
Aeromonas sp.	2	2	
Arachnia propionica	2	2	
Bacillus alvei	2	2	
Bacillus anthracis*	2	2/3	BMBL, vaccination recommended
Bacteroides sp.	2	2	
Bartonella sp.	2	3	
Bordetella sp.	2	2	
Bordetella pertussis	2	2/3	BMBL
Borrelia sp.	2	2	
Brucella sp.*	2/3	3	BMBL
Campylobacter fetus var. jejuni	2	2	BMBL
Campylobacter sp.	2	2	
Chlamydia psittaci	2	3	BMBL
Chlamydia pneumoniae	2/3	2	BMBL
Chlamydia trachomatis	3	3	
Clostridium botulinum*	2/3	2	BMBL
Clostridium tetani	2	2	BMBL
Corynebacterium diphtheriae	2	2	BMBL

Corynebacterium equi/ Rhodococcus equi	2	2	
Corynebacterium haemolyticum	2	2	
Corynebacterium pseudotuberculosis	2	2	
Corynebacterium pyogenes	2	2	
Corynebacterium renale	2	2	
Enterobacteriaceae all other	2	2	
Erysipelothrix rhusiopathiae	2	2	
Escherichia coli	2	2	
Escherichia coli K12 derivative	1	1	
Francisella tularensis*	2/3	3	BMBL
Fusobacterium sp.	2	2	
Haemophilus sp.	2	2	
Klebsiella sp.	2	2	
Legionella pneumophilia	2/3	2	BMBL
Leptospira interrogans all serovars	2	2	BMBL
Listeria sp.	2	2	
Moraxella sp.	2	2	
Mycobacterium avium	2	2	
Mycobacterium bovis	2	3	BMBL
Mycobacterium leprae	2	2	BMBL
Mycobacterium sp.	2	2	BMBL
Mycobacterium tuberculosis	2/3	2/3	BMBL
Mycoplasma sp.	2	2	
Neisseria gonorrhoeae	2/3	2	BMBL
Neisseria meningitidis	2/3	2	BMBL
Nocardia sp.	2	2	

Pasteurella sp.	2	2	
Pseudomonas mallei	2/3	3	BMBL
Pseudomonas testosteroni/ Comamonas testosteroni	2	2	
Rhodococcus (Coryne.) equi	2	2	
Salmonella sp.	2	2	BMBL
Salmonella typhi	2/3	2	BMBL
Shigella sp.	2	2	BMBL
Staphylococcus sp.	2	2	
Streptococcus sp.	2	2	
Streptobacillus moniliformis	2	2	
Streptomyces somaliensis	2	2	
Treponema pallidum	2	2	BMBL
Vibrio sp.	2	2	BMBL
Yersinia pestis*	2/3	3	BMBL, immunization recommended
BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 6th Edition			
V - Vaccination is recommended for personnel			
***** - Select agents			

Fungal Agent	BSL	ABSL	Comments
Blastomyces dermatitidis	2	2	BMBL
Coccidioides immitis*	2/3	2	BMBL
Cryptococcus neoformans	2	2	BMBL
Epidermophyton - pathogenic sp.	2	2	BMBL
Histoplasma capsulatum	2/3	2	BMBL
Microsporum - pathogenic sp.	2	2	BMBL
Paracoccidioides brasiliensis	2	2	
Sporothrix schenckii	2	2	BMBL
Trichophyton - pathogenic sp.	2	2	BMBL
Candida albicans	2	2	
Miscellaneous Molds	2		BMBL
BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 6th Edition			
V - Vaccination is recommended for personnel			
***** - Select agents			

Parasitic Agents	BSL	ABSL	Comments
Anaplasma sp.	2	2	
Ascaris sp.	2	2	BMBL
Coccidia sp.	2	2	BMBL
Cryptosporidia sp.	2	2	BMBL
Echinococcus granulosus	2	2	BMBL
Ehrlichia sp.	2	2	
Entamoeba sp.	2	2	BMBL
Enterobius sp.	2	2	BMBL
Fasciola sp.	2	2	BMBL

Giardia sp.	2	2	BMBL
Haemobartonella sp.	2	2	
Hymenolepis nana	2	2	BMBL
Leishmania sp.	2	2	BMBL
Leukocytozoon sp.	2	2	
Naegleria sp.	2	2	
Plasmodium sp.	2	2	BMBL
Sarcocystis sp.	2	2	BMBL
Schistosoma sp.	2	2	BMBL
Strongyloides sp.	2	2	BMBL
Taenia solium	2	2	
Toxocara canis	2	2	
Toxoplasma sp.	2	2	BMBL
Trichinella spiralis	2	2	BMBL
Trypanosoma sp.	2	2	BMBL
BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 6th Edition			
V - Vaccination is recommended for personnel			
***** - Select agents			

Rickettsial Agents	**BSL**	**ABSL**	**Comments**
Coxiella burnetii*	2/3	3	BMBL
Rickettsia akari	2/3	2/3	
Rickettsia australis	2/3	2/3	BMBL
Rickettsia canadensis	2/3	2/3	BMBL
Rickettsia conorii	2/3	2/3	BMBL
Rickettsia prowazekii*	2/3	2/3	BMBL
Rickettsia rickettsii*	2/3	2/3	BMBL

Rickettsia sibirica	2/3	2/3	BMBL
Rickettsia tsutsugamushi	2/3	2/3	BMBL
Rickettsia typhi (R. mooseri)	2/3	2/3	BMBL
Rochalimaea quintana	2	2	
Rochalimaea vinsonii	2	2	
Spotted Fever Group - other	2/3	2/3	
BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 6th Edition			
V - Vaccination is recommended for personnel			
***** - Select agents			

Viral Agents	**BSL**	**ABSL**	**Comments**
Adenoviruses	2	2	
Adenoviruses - animal - all	2	2	
Aleutian Disease Virus	2	2	
Arboviruses - certain	2	2	BMBL
Arboviruses - certain	3	3	BMBL
Arboviruses - certain	4	4	BMBL
Arenaviruses - certain	3	3	BMBL
Arenaviruses - certain	4	4	BMBL
Avian Erythroblastosis Virus	2	2	
Avian Leucosis Virus	2	2	
Avian Lymphomatosis Virus	2	2	
Avian Myeloblastosis Virus	2	2	
Bovine Encephalomyelitis Virus	2	2	
Bovine Leukemia Virus	2	2	
Bovine Resp. Syncytial Virus	2	2	

Bovine Rhinotracheitis (IBR)	2	2	
Cache Valley Virus	2	2	BMBL
Canine Hepatitis Virus	2	2	
Canine Distemper Virus	2	2	
Caprine Arthritis	2	2	
Coxsackie A & B Viruses	2	2	
Cytomegaloviruses	2	2	
Encephalomyelitis Virus*	2	2	
Echovirus	2	2	
Dengue Virus	2	3	BMBL
Encephalomyocarditis Virus	2	2	
Epidemic Diarrhea Infant Mice	2	2	
Epstein-Barr Virus	2	2	
Feline Leukemia Virus	2	2	
Feline Sarcoma Virus	2	2	
Filoviruses	2	2	
Flanders Virus	2	2	BMBL
Gibbon Ape Lymphosarcoma	2	2	
Hart Park Virus	2	2	BMBL
Hemorrhagic Fever Agents*	2	2	
Hep A Virus, Hep E Virus	2	2	BMBL
Hep B Virus, Hep C Virus	2	2	BMBL
Virus, Hepatitis D Virus	2	2	BMBL
Herpesvirus - other	2	2	
Herpesvirus ateles	2	2	
Herpesvirus saimiri	2	2	
Herpesvirus Simiae (B-virus)	3	3	BSL-2, -3 or -4 depending on activity, BMBL

Human Herpesviruses	2	2	BMBL
Hog Cholera Virus	2	2	
Human T-Cell Leukemia Virus I & II	2	2	
Infectious Bronchitis Virus	2	2	
Influenza Virus	2	2	BMBL
Influenza Virus Virulent Avian	3	3	
K (Rate) Virus	2	2	
Lactic Dehydrogenase Elevating	2	2	
Langat Virus	2	2	BMBL
Laryngotracheitis Virus	2	2	
Lassa Virus*	4	4	BMBL
Low Risk Oncogenic Viruses	2	2	
Lymphocytic Choriomeningitis Virus	2/3	2/3	BMBL
Marburg Virus*	4	4	BMBL
Measles Virus	2	2	
Meningopneumonitis Virus	2	2	
Mouse Encephalomyelitis Virus	2	2	
Mouse Hepatitis Virus	2	2	
Mouse Leukemia Virus	2	2	
Mouse Pneumonia Virus	2	2	
Mumps Virus	2	2	
Myxomatosis Virus	2	2	
Newcastle Disease Virus	2	2	
Newcastle Disease Virus (VVND)	2	2	
Non-Defective Adenovirus 2SV40 HYB	2	2	

Papilloma Virus Shope	2	2	
Parainfluenza Virus	2	2	
Poliovirus - all types	2	2	BMBL
Polyoma Virus	2	2	
Poxvirus alastrim	2	2	
Poxvirus monkey pox	3	3	
Poxvirus - Smallpox*			restricted use by WHO
Poxvirus sp.	2	2	BMBL
Pseudorabies Virus	2	2	
Rabies Virus	2/3	2/3	BMBL
Reovirus sp.	2	2	
Respiratory Syncytial Virus	2	2	
Retroviruses, including HIV & SIV	2/3	2/3	BMBL
Rhinovirus sp.	2	2	
Rous Sarcoma Virus	2	2	
Rubella Virus	2	2	
Simian Virus - other	2	2	
Simian T-Cell Leukemia Virus	2	2	
Sindbis Virus	2	2	
Slow Viruses	2	2	
Tensaw Virus	2	2	
Tick-Borne Encephalitis Complex	4	4	
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru, and related agents	2	2	BMBL
Turlock Virus	2	2	
Vaccinia Virus	2	2	

Venezuelan Equine Encephalitis*	3	3	
Vesicular Stomatitis - lab adapted	2	2	BMBL
Vesicular Stomatitis Virus	3	3	BMBL
Woolly Monkey Fibrosarcoma	3	3	
Yaba Virus	2	2	
Yellow Fever Virus 17D Strain*	2	2	BMBL
Yellow Fever Virus Except 17D*	3	3	BMBL
BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 6th Edition			
V - Vaccination is recommended for personnel			
***** - Select agents			

Appendix 2: Biosafety Levels for Commonly Used Mammalian Cell Liens

The table below is not an exhaustive list; if you work with a mammalian cell line not listed, please perform a risk assessment and consult with the EH&S Department to determine the appropriate Biosafety Level.

Table 5. Biosafety Levels for Commonly Used Mammalian Cell Lines

Mammalian Cell Line	Common Abbreviation	BSL
293 [HEK-293] Human embryonic kidney epithelial cells	293 or HEK293	2
293T Human embryonic kidney epithelial cells with SV40 T-antigen (highly transfectable)	293T	2
A549 Human lung epithelial cells (carcinoma)	A549	1
COS-7 African green monkey kidney fibroblast cells with SV40 T-antigen	COS-7	2
HeLa Human cervical epithelial cells (adenocarcinoma)	HeLa	2
Hep G2 Human liver epithelial cells (hepatocellular carcinoma)	HEPG2	1
HK-2 Human adult kidney proximal tubule epithelial cells (papilloma)	HK-2	2
MCF7 Human mammary gland epithelial cells (adenocarcinoma)	MCF7	1
NIH/3T3 Mouse embryonic fibroblast cells	NIH/3T3	1

RAW 264.7 Mouse macrophage cells (from Abelson murine leukemia virus-induced tumor)	RAW 264.7	2
THP-1 Human monocyte cells (acute monocytic leukemia)	THP-1	1
Vero African green monkey kidney epithelial cells	Vero	1

Appendix 3: Summary of Biosafety Levels (BSL-3, BSL-4, & ABSL-3)

Biosafety Level 3 and 4 (BSL-3 and BSL-4) require special containment laboratories or facilities, which are not available at CSUS. The summary below is provided to briefly demonstrate the difference requirements for BSL-3 and BSL-4.

Table 6. Summary of Biosafety Levels (BSL-3, BSL-4, & ABSL-3)

Biosafety Level 3 (BSL-3) – Not Available at CSUS	
Agents:	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences
Practices:	BSL-2 practice **plus**: Controlled access; decontamination of all waste; decontamination of lab clothes before laundering; baseline serum
Safety Equipment: (Primary Barriers)	Biological Safety Cabinet (BSC) or other physical containment devices used for all manipulations of agents as well as PPE such as protective lab clothing, gloves, and respiratory protection, as needed
Facilities: (Secondary Barriers)	BSL-2 facility **plus**: Physical separation from access corridors; self-closing, double door access; exhaust air not recirculated; negative airflow into laboratory
Biosafety Level 4 (BSL-4) – Not Available at CSUS	
Agents:	Dangerous and exotic biological agents that pose a high individual risk of life-threatening disease that may be transmitted via the aerosol route and for which there is no available vaccine or therapy
Practices:	BSL-3 practices **plus**: Controlled access with documentation of date and time of all persons entering and leaving the laboratory; inventory system for agents; all personal clothing or jewelry (except eyeglasses) must be removed in the outer clothing changing room; all persons entering lab use laboratory clothing including: undergarments, pants, shirts, socks, jumpsuits, shoes, and gloves; all persons leaving the lab must take a personal body shower; documented daily inspections of essential containment and life support systems; supplies and materials must enter the lab through a dunk tank, previously decontaminated double-door autoclave, fumigation chamber, or airlock; the outer door of the autoclave or fumigation chamber

	is not opened until the autoclave, fumigation chamber, or airlock has been operated through a successful decontamination cycle
Safety Equipment: (Primary Barriers)	Class III Biological Safety Cabinet (BSC) or Class II BSC with a full-body air-supplied positive-pressure personnel suit to completely isolate the lab worker from aerosolized infectious materials
Facilities: (Secondary Barriers)	BSL-3 facility **plus**: Often a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems, for both solid and liquid waste, to prevent the release of hazardous biological agents into the surrounding community and the environment; additional secondary barriers dependent on whether a "cabinet" or "suit" laboratory (see primary barriers above)
Animal Biosafety Level 3 (ABSL-3) – Not Available at CSUS	
Agents:	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects
Practices:	ABSL-2 practices **plus**: Controlled access; decontamination of clothing before laundering; cages decontaminated before bedding removed; disinfectant footbath as needed
Safety Equipment: (Primary Barriers)	ABSL-2 equipment **plus**: Containment equipment for housing animals and cage dumping activities; Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols; respiratory protection
Facilities: (Secondary Barriers)	ABSL-2 facility **plus**: Physical separation from access corridors; self-closing, double door access; sealed penetrations; sealed windows; autoclave available in facility

Appendix 4: Biosafety Cabinets

Biological Safety Cabinets (BSCs), or biosafety cabinets, are the primary means of containment for working safely with potentially infectious materials and can be used for manipulation of sterile cultures. BSCs are designed to provide personnel and environmental protection when appropriate practices and procedures are followed. Lack of appropriate practices and procedures (e.g., poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique) compromise the containment capability of a BSC.

There are three types of BSCs as defined by CDC/NIH's *Biosafety in Microbiological and Biomedical Laboratories*. EH&S should be consulted for selection, purchase, installation, and use of BSCs on campus.

1. Testing and Certification of BSCs

BSCs must be tested and certified after installation, alterations, or maintenance, and at least annually thereafter to ensure continued, proper operation. Testing and certification of BSCs will be performed by an outside contractor. Contact EH&S for more information. Tests are conducted in accordance with the most recent edition of NSF International Standard No. 49, Class II (Laminar Flow) Biohazard Cabinetry.

2. Types of Biological Safety Cabinets

- Class I BSC - The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in function to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. The Class I BSC is not commonly used on campus.
- Class II BSC (Types A, B1, B2 and B3) - Class II cabinets are designed for work involving microorganisms assigned to Biosafety Levels 1, 2, and 3. These cabinets provide personnel, environmental, and product protection. They provide the microbe-free work environment necessary for cell culture propagation and may be used for nonvolatile chemotherapeutic drug preparation.
- Class III BSC - The Class III biological safety cabinet is designed for work with Biosafety Level 4 microbiological agents and provides maximum protection to the operator and the environment.
- Horizontal and Vertical Laminar Flow "Clean Bench" - Horizontal and vertical laminar-flow clean-air benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices provide only product protection.

- HEPA filters do not absorb chemical vapors or gases, therefore BSCs cannot be used for protection against gases and vapors as there is potential for buildup of hazardous concentrations within the cabinet. Experiments that produce chemical vapors or gases should be performed in a certified chemical fume hood.

3. Procedures for Use of BSCs

Start-up Procedure

1. Turn off ultraviolet light, if so equipped.
2. Turn on all blowers and cabinet illumination lights.
3. Allow five minutes of operation to purge system and check flow alarm system audio and visual alarm function, if so equipped.
4. Decontaminate readily-accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.

Working in the BSC

1. Check the Magnehelic gauge regularly for any indication of a problem.
2. Plan your work prior to starting.
3. Minimize the storage of materials in and around the BSC.
4. Always leave the BSC running while working in the cabinet.
5. Appropriate PPE should be worn as indicated by the risk assessment; this should include, but is not limited to, laboratory coat worn buttoned over clothing and disposable gloves.
6. Adjust the stool height to an ergonomic position that allows proper back and feet support and so the user's face is above the front opening of the cabinet. Ensure work can be conducted with the user's arms raised slightly above the front grille of the cabinet (not resting on the grille).
7. 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 behind you, and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.

Operational Directions

1. Before using, wipe readily-accessible interior work surfaces with 70% ethanol or another disinfectant suitable to meet the requirements of the activity. Wipe off the surfaces of all materials and containers you need for your procedures with 70% ethanol and place them in cabinet.
2. Place objects as far back in the cabinet as practical. Do not place objects over the front air intake grille. Do not block the rear exhaust grille.

3. Segregate contaminated and clean items. Work from "clean to dirty."
4. Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discard. Do not use vertical pipette discard canisters on the floor outside cabinet.
5. Open flames are not required or recommended in the BSC. They create turbulence in airflow and will compromise sterility; heat buildup may damage the filters.
6. Move arms slowly when removing or introducing new items into the BSC.
7. If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge or blender), place equipment in the back one-third of the cabinet; stop other work while equipment is operating.
8. Protect the building vacuum system from biohazards by connecting the aspirator bottles or suction flasks to an overflow collection flask and an in-line HEPA filter.
9. Clean up all spills in the cabinet immediately. Wait 10 minutes before resuming work.

Shutdown Procedures

1. When work is finished, remove all materials from the interior work area; decontaminate them with an appropriate surface disinfectant, as needed.
2. Wipe all readily-accessible interior surfaces with 70% ethanol or other appropriate disinfectant.
3. Allow five minutes of operation to purge the system.
4. Turn off the cabinet blower.
5. Turn on the ultraviolet light, if so equipped.
6. Remove gloves and lab coat, and wash hands thoroughly before leaving the laboratory.

Appendix 5: Biohazardous Spill Clean-up Protocol

Spills of BSL-2 material that result in injury to personnel or release greater than 1 liter must be reported to your lab supervisor and EH&S. Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards must have a basic biohazardous spill kit ready to use at all times. Materials should be easily accessible to everyone in the lab. If your BSL-2 lab does not have a spill kit, please contact EH&S.

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 911 if necessary, and proceed with common sense. Call EH&S to request the spill response team if additional assistance is required, especially if the spill outgrows the resources in the laboratory.

1. BSL-1 Spills

- Notify others in the area to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing and wash exposed skin with soap and water.

Clean-up of BSL-1 Spill

- Don disposable gloves, goggles, lab coat, and closed-toe shoes. Face shields are recommended for spills with potential for splashes.
- Pick-up any pieces of broken glass or other solid materials in the spill with forceps and place in broken glass waste receptacle.
- Cover spill with paper towels or other absorbent material, pour disinfectant around the spill allowing it to mix with the spilled material. Allow at least 10 minutes of contact time (assuming sodium hypochlorite solution is being utilized).
- Mechanically scoop up the absorbed spill using scoops or cardboard.
- Discard all disposable materials used to clean-up the spill into municipal waste receptacle.
- Wash hands with soap and water.

2. BSL-2 Spills

- Most agents used at BSL-2 are not airborne pathogens. However, if agent poses an inhalation risk, quickly leave the room. Notify others to leave. Close door, and post with a warning sign.
- If liquid spill has contaminated clothing, remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag.
- Wash all exposed skin with soap and water.

- Notify Principal Investigator or lab supervisor of incident.

Clean-up of BSL-2 Spill

- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory, if applicable.
- Assemble clean-up materials from spill kit and lab (disinfectant, paper towels or other absorbent, biohazard bags, forceps, etc.).
- Don disposable gloves, goggles, lab coat, closed-toe shoes, and shoe covers, if necessary. Face shield should also be utilized for large spills with potential for splashes.
- Depending on the nature of the spill, it may be advisable to wear an N-95 filtering face piece. The N-95 face piece should only be worn if there is an airborne hazard present, and only by those who have met the requirements of the CSUS Respiratory Protection Program.
- Pick up any sharp objects with forceps or tongs and discard in a biohazardous sharps container. Smaller pieces of glass may be collected with cotton or paper towels held with forceps.
- Cover the area of the spill with paper towels or other absorbent material sufficient to soak up the liquid, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. *Allow at least 20 minutes of contact time (assuming sodium hypochlorite solution is being utilized).*
- Using mechanical means, scoop up the absorbed spill material and discard in red biohazard bags.
- Spray and wipe surrounding areas (where the spill may have splashed) with disinfectant and wipe up with paper towels. Place all contaminated paper towels and any contaminated clothing into a biohazard bag.
- Remove and discard gloves, then wash hands and exposed skin areas with soap and water.
- All BSL-2 waste is to be disposed of at the biohazard waste collection site of the TSC loading dock.

3. Spill within a Biological Safety Cabinet (BSL-2)

- Leave the BSC blower on and begin clean-up immediately.
- While wearing lab PPE, cover the spill area with paper towels or disinfectant-soaked paper towels. Do not place your head in the cabinet to clean the spill, keep your face behind the view screen.

- If necessary, flood the work surface as well as the drain pans and catch basins below the work surface with disinfectant. Be sure the drain valve is closed before flooding the area under the work surface.
- Wipe cabinet walls, work surfaces, and inside the view screen with disinfectant.
- Lift the front intake grill and work surface; wipe all surfaces with disinfectant. Be sure no paper towels or soiled debris are blown into the area under the spill tray.
- If the work surface, as well as drain pans and catch basins under the work surface have been flooded, soak up disinfectant on work surface. Place container under the drain valve and drain the disinfectant under the work surface into a container.
- Wipe the areas under the work surface to remove residual disinfectant.
- Collect all clean-up materials and used gloves in a biohazard bag.
- Wash hands and exposed skin with soap and water.
- Notify Principal Investigator or lab supervisor of incident.
- All BSL-2 waste is to be disposed of at the biohazard waste collection site of the TSC loading dock.

Appendix 6: Decontamination

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

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

- Type of biohazardous agent(s), concentration, and potential for exposure
- Physical and chemical hazards to products, materials, environment, and personnel

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

- Heat
- Liquid chemicals

1. Heat

In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat (steam sterilization in an autoclave) is a better heat transfer to and into the biological material resulting in overall shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 250-270°F (121-132°C) 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 320-338°F (160-170°C) for periods of 2 to 4 hours. Note: Sac State must obtain a permit from the state Department of Health Services (DHS) to be a medical waste treatment facility. This requires inspection and certification of specific autoclaves that will be used for medical waste treatment. Currently, Sac State does not have any permitted autoclaves.

2. 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 biological agent – Microbial agents can be classified according to increasing resistance to disinfectants (see Table 7).
- Nature of surface being disinfected – Porous or smooth; more porous and rough surfaces require increased concentration and/or contact time.
- Number of microorganisms present – Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Presence of organic material – The proteins in organic materials, such as blood, bodily fluids, and tissue, can prevent or slow the activity of certain disinfectants.
- Duration of exposure (i.e., contact time) and temperature – Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.
- Toxicity and reactivity of chemical disinfectant – Many disinfectants pose significant health hazards to humans and may chemically react with the samples to be disinfected.

Table 7. Resistance to Chemical Disinfectants (Examples)

		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 choleraesuis
	FUNGI	Trichophyton sp. Cryptococcus sp. Candida sp.
	NON-LIPID or SMALL VIRUSES	Poliovirus Coxsackievirus Rhinovirus
	MYCOBACTERIA	Mycobacterium tuberculosis Mycobacterium bovis
Most Resistant	**BACTERIAL SPORES**	Bacillus subtilis Clostridium sporogenes

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalis, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are discussed below:

- **Alcohols:** Ethyl or isopropyl alcohol in concentration of 10% to 70% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores. Concentrations above 70% are less effective.
- **Formalin:** Formalin is 37% solution of formaldehyde in water. Dilution of formaldehyde to 5% results in an effective disinfectant. Formaldehyde is a human carcinogen and creates respiratory problems at low levels of concentration.
- **Glutaraldehyde:** Although chemically related to formaldehyde, this compound is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehydes are irritating to the eyes, nasal passages, and upper respiratory tract. They should be used always in accordance with the instructions on the label and appropriate personal protective equipment.
- **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 to disinfect contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including Mycobacterium tuberculosis, fungi, and lipid-containing viruses. They are not active against spores or non-lipid viruses.
- **Quaternary Ammonium Compounds (Quats):** Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. They are less active against Gram-negative bacteria and are not active against non-lipid-containing viruses. Quats are easily inactivated by organic materials, anionic detergents, or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants and cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general cleaning.
- **Halogens (Chlorine and Iodine):**
    - Chlorine-containing solutions have broad spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a

satisfactory disinfectant solution. Diluted solutions should be kept no longer than one week, assuming they are maintained in closed containers and protected from light. It is recommended to use freshly-prepared solutions for spill clean-up purposes. Chlorine-containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilizers since they inactivate bacterial spores.

- Iodine has similar properties to chlorine. Iodophors (iodine in combination with a surfactant) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

It is important to note that the U.S. Environmental Protection Agency and California Environmental Protection Agency define antimicrobials such as disinfectants, sanitizers, and bacteriostats as pesticides. Worker safety requirements for pesticides are similar to those for other hazardous materials administered by Cal-OSHA.

When using a chemical disinfectant, remember that you are using a potentially toxic chemical that could be a corrosive, flammable solvent and/or a carcinogen. Wear PPE as indicated on the product container and Material Safety Data Sheet (MSDS). If you must prepare a dilution of the disinfectant, do so whenever possible in a chemical fume hood or in a well-ventilated area. If you are working with mixed solutions, check the MSDS to ensure that any incompatible chemical reaction will not result.

Avoid using concentrated or undiluted solutions of your disinfectant to speed up the inactivation process. Undiluted chemicals may adversely affect the surface being cleaned. This is especially significant when working with bleach, which is a very strong corrosive. Some disinfectants will leave a residue. Rinse the cleaned area with distilled water to avoid adverse effects on the experiment after allowing sufficient contact time. This is especially important in tissue culture rooms where a cell line can be destroyed by disinfectant residue left on equipment.

Allow sufficient contact time after applying the disinfectant. Do not apply a disinfectant and immediately remove it from the contaminated surface, as the contact time will be too brief to ensure that the surface has been thoroughly disinfected. When cleaning a spill of concentrated

material or if the disinfectant must act on an uneven surface, allow extra time for the disinfectant to act.

Appendix 7: Transportation and Shipping

All biological materials should be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release or endangerment to the public or environment. The shipment of diagnostic and clinical specimens, biological products, infectious agents, and recombinant DNA molecules is regulated by Department of Transportation (DOT) for packages shipped by road and International Air Transport Association (IATA) for packages shipped by air. This includes specific procedures for the correct packing and packaging of these materials, necessary documentation, and labeling and permits. Contact EH&S for assistance shipping these materials.

1. Transportation Outside of the Laboratory On-Campus

- Biohazardous agents must be properly handled, contained, and labeled during transport between locations to prevent accidental exposure to unsuspecting persons outside of the laboratory.
- Biohazardous agents must be placed in securely closed primary containers. The exterior of the primary container should be decontaminated prior to transportation.
- The primary container should be placed in a covered, leakproof, shatterproof secondary container. The secondary container should be labeled with the biohazard symbol, the biohazardous agents present, and the lab of origin. If it is transported by vehicle, the name and telephone number of the PI or other responsible person(s) must be included on the outside of the secondary container.

2. Shipment Off-Campus (Domestic Shipment)

- All domestic and international shipments of infectious substances require the use of packaging that has been tested and certified to carry such material. Certified packaging will have markings on the outside indicating that it has met performance tests.
- When transporting infectious agents, the shipper is responsible for the proper packing of dangerous goods and must pack biological agents as infectious substances (Packing Instruction 602, IATA-DGR) or diagnostic specimens (Packing Instruction 650, IATA-DGR). The following are packing instructions for infectious substances:
    - Primary and Secondary Containers
        - The specimen will be placed in a securely closed, watertight primary container. Stoppers and screw-capped tubes will be secured with waterproof tape.
        - The contents of the primary container will not exceed 50 mL.

        - The exterior of the primary container will be decontaminated prior to transportation.
        - A biohazard label will be placed on the exterior of the primary container.
        - One (or more) primary container(s) may be placed within the secondary container as long as the total volume of the specimen does not exceed 50 mL.
        - The absorbent material used within the secondary container must be sufficient to absorb the contents of the primary container(s), if it should leak.
        - The secondary container must be free of contamination and labeled with the same symbol as the primary container.
    - Outer Container
        - This container will be made of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.
        - The interior of the outer container may be filled with coolant material such as ice or dry ice. If ice or dry ice is used, additional shock absorbent material will be added and positioned in a manner that allows protection of the specimen should the ice or dry ice melt or sublimate. The dry ice should be placed outside of the secondary container in the outer container.
        - The exterior will be labeled with the special sticker depicted in Figure 2.
        - Figure 2. Etiologic Agents
        - Prior to transport, the outer container should be sealed or secured in a manner so as to make it leak-proof should the container be placed on its side.
        - The package will be decontaminated before shipment.

3. International Shipments

- All domestic and international shipments of infectious substances require the use of packaging that has been tested and certified to carry such material. Certified packaging will have markings on the outside indicating that it has met performance tests.
- A statement should be included in the additional handling information that states, "Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made."
- The shipper should include the name and telephone number of the person responsible for the shipment.

- Diagnostic specimens being shipped for the purpose of initial diagnosis are excluded from the regulations. However, diagnostic specimens known, or thought likely, to contain infectious substances are included.

4. Receipt

- Upon receipt of any packaged specimens, immediately check for leakage or damage.
- If leaking:
    - Isolate the package either in a Class II biological safety cabinet or in a leak-proof, sealed container. Add disinfectant and dispose of as medical waste.
    - Keep unauthorized personnel away from the package.
- The package should be opened in the laboratory on an easily cleaned, water-resistant surface.