

BIOSAFETY/BIOHAZARD MANUAL for CALIFORNIA STATE UNIVERSITY, FULLERTON ACADEMIC LABORATORIES and CLASSROOMS

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I. PURPOSE OF THE MANUAL

This manual provides the procedures for the safe handling and disposal of biohazards at CSUF. These requirements are based on present knowledge and may change as research and teaching needs expand and new legislation is written.

NOTE: If your work also includes the use of hazardous chemicals or radiation, please check with the Radiation Safety Officer and the Chemical Hygiene Officer for the specifics of the safe handling and disposal of these materials. See Addendum A for mixed hazardous waste hierarchy.

II. RESPONSIBILITY IN THE USE OF BIOHAZARDS

A. Principal Investigator or Course Instructor will:

1. Supervise and be responsible for all lab personnel, including students;
2. Be well-versed on the use and biohazard potential of all lab materials;
3. Clearly label all biohazards and biohazard work areas;
4. Train lab personnel regarding the use of all biohazards in his or her lab;
5. Develop emergency procedures specific to the biohazards in his or her lab;
6. Train lab personnel regarding emergency procedures;
7. Include the cost of disposal and containment of biohazards into grants, when possible.

B. Biosafety Officer will:

1. Coordinate and develop the campus biohazard program;
2. Provide information on the handling and disposal of biohazards;
3. Organize disposal of biohazards;
4. Train personnel including students in basic safety techniques, as needed;
5. Respond to emergency situations.

C. Technicians, teaching assistants, laboratory assistants will:

1. Aid in training of lab personnel including students in laboratory procedures;
2. Report violations to his or her supervisor;
3. Ensure proper labeling;
4. Understand emergency procedures and be able to direct others in emergency situations.

III. UNIVERSITY BIOSAFETY COMMITTEE (UBC)

A. University Biosafety Committee (UBC) purpose:

1. Review all programs involving recombinant DNA¹, infectious disease agents, and any other dangerous biological materials.
2. Approve such programs based on their hazard and proposed containment procedures.
3. The committee shall meet periodically to review biosafety issues as the need arises.
4. The committee will be composed of at least 4 faculty members¹ who currently use or are knowledgeable about biological hazards. The University Biosafety Officer (described below) will be a permanent member.

B. University Biosafety Officer (BSO) responsibility:

1. Reviews current and new laws affecting the use of biohazards.
2. Is apprised of and reviews all proposed work with biohazards.
3. Serves as liaison between the researcher and the UBC.
4. Serves as a safety consultant for on-going research programs.
5. Keeps all committee records.

¹NIH has established specific guidelines for institutes which are engaged in recombinant DNA research. Among those guidelines is the establishment of an Institutional Biosafety Committee whose membership is clearly defined. If any researcher at CSUF is engaged in any level of recombinant DNA research which requires such a committee, the University Biosafety Committee membership must reflect the NIH requirements. See Appendix B for a complete description.

IV. BIOHAZARDS

A. DEFINITION

Infectious agent (Biohazard): a type of micro-organism, bacteria, mold parasite or virus which normally causes, or significantly contributes to the cause of, increased morbidity or mortality of human beings.

For the purposes of this manual, this description will be appropriate at CSUF for the protection of all persons, animals and plants that would be affected by the use or misuse of any biohazards used on this campus.

1. Biohazards include:
 - a. Biologically infectious (human pathogenic) agents ,
 - b. Animals and their waste, experimentally or naturally infected with infectious agents,
 - c. Any object contaminated with infectious agents,
 - d. Sharps (syringes with attached needles, needles, razor blades, glass slides, cover slips and disposable glass Pasteur pipets) contaminated with infectious agents.

B. PROCEDURES FOR HANDLING BIOHAZARDS

1. Good laboratory practices must be followed at all times. However, working with infectious material requires the following additional precautions.
 - a. Limit access to areas where experiments with infectious agents are in progress.
 - b. Clearly label areas where biohazards are in use and designate specific areas where biohazards are routinely used, using this symbol (black on red background):



- c. Wear lab coat, gloves and safety glasses to prevent contamination from the infectious material, and remove them when leaving the work area.
- d. Decontaminate work surfaces once per day and after any spill of viable material.
- e. Use only mechanical pipetting devices; **mouth pipetting is prohibited.**
- f. Eating, drinking and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators designated for this purpose and should be located outside the work area.
- g. Wash hands after handling viable materials and animals and before leaving the lab.
- h. Perform all procedures carefully to minimize the creation of aerosols.
- i. Transport contaminated materials in a red biohazard bag which has been placed in a labeled, leak-proof container.
- j. To eliminate finger sticks, do not recap needles.

2. Hazard Determination

a. Persons who work with materials which have special hazards must follow any additional precautions as outlined by the University Biosafety Committee.

b. Lab Biosafety Level Criteria

The following guidelines² will be used by all laboratory personnel and the University Biosafety Committee to determine the proper practices, safety equipment and facilities applicable to the hazards present.

- i. Biosafety Level 1 is appropriate for undergraduate and secondary educational training and teaching laboratories and/or other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans.
- ii. Biosafety Level 2 is applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity.
- iii. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences.
- iv. Biosafety Level 4 is applicable to work with dangerous and exotic agents which pose a high individual risk of life-threatening disease.
- v. Summary:

Biosafety Level	Practices and Techniques	Safety Equipment	Facilities
1	Standard microbiological practices	None; primary containment provided by adherence to standard laboratory practices during open bench operations.	Basic
2	Level 1 practices plus: Laboratory coats; decontamination of all infectious wastes; limited access; protective gloves and biohazard warning signs as indicated.	Partial containment equipment (i.e., Class I or II Biological Safety Cabinets) used to conduct mechanical manipulative procedures that have high aerosol potential that may increase the risk of exposure to personnel.	Basic
3	Level 2 practices plus: special laboratory clothing; controlled access.	Partial containment equipment used for all manipulations of infectious material	Containment
4	No facilities available on this campus at this time.	Maximum containment equipment.	Maximum Containment

²These guidelines are written by the U.S. Department of Health and Human Services, Public Health Service, CDC and NIH. See Reference 1.

V. DISPOSAL OF BIOLOGICAL WASTE

NOTE: There is often confusion about what constitutes “biohazardous waste” versus “medical waste.” A clear distinction must be made because the regulations dictate different means of disposal of these two waste streams.

A. DEFINITIONS OF WASTE

1. **Biohazardous Waste** is:

- a. Laboratory waste generated from human or animal specimen cultures from medical and pathology labs, or cultures and stocks of infectious agents from research labs,
- b. Human surgery specimens or tissues removed at surgery, which are suspected by the attending physician of being contaminated with infectious agents known to be contagious to humans,
- c. Animal parts, excrement or fluid (including blood) that are known or suspected of being contaminated with infectious agents known to be contagious to humans.

2. **Medical Waste** is:

- a. Waste created in the diagnosis, treatment or immunization of humans or animals, or research pertaining to such activities, AND is also biohazardous waste.

TO CLARIFY: At CSUF, biohazardous waste is generated in some research laboratories (as bacterial cultures, some tissue culture stocks, etc.), but (except for human anatomy and immunology labs) “medical waste” is not generated. The primary location where “medical waste” is generated is the Student Health Center and some Training Rooms in Kinesiology, where patients are treated. However, ALL human samples will be treated as “medical waste.”

B. HANDLING BIOHAZARDOUS (INFECTIOUS) WASTE

1. Items Which Require Containment:

- a. Contaminated Sharps (syringes with attached needles, needles, razor blades, glass slides, cover slips and disposable glass Pasteur pipets) -- must be placed in labeled (red, with Biohazard symbol) puncture-proof containers (provided and disposed of by Biosafety Officer). **NO CHEMICALS OR RADIOACTIVE MATERIAL!**
- b. Animal carcasses, contaminated with infectious agents, in part or whole³, must be either:
 - i. Placed in red biohazard bags⁴ (provided by Biosafety Officer), or
 - ii. Placed directly into red 5 gallon biohazard containers (with 2-inches of sawdust in the bottom to absorb any residual liquid), preferably lined with red biohazard bags.

³ See Appendix C for Formaldehyde/Preserved Carcasses.

⁴ NOTE: Red biohazard bags are NOT AUTOCLAVABLE! These are for disposal infectious animal or human waste that is given to the Biosafety Officer for disposal.

2. Items Which Require Treatment:
 - a. Cultures of the following items, and the dishes, pipets and containers used for their containment or transfer, must be completely inactivated by autoclaving before disposal of solids in the normal trash, or liquids in the lab drain, or reuse of non-disposable glassware. See Autoclaving, Section V, D and Appendix E.
 - i. Pathogenic bacteria cultures, tissue cultures, molds, fungi, parasites, viruses or biologically produced toxins.
 - b. Contaminated liquid animal blood, blood products, and body fluids. -- these items must be autoclaved before their disposal in the laboratory garbage disposal.
 - c. Any other object or substance contaminated with infectious or pathological agents must be autoclaved before disposal or decontaminated with a reliable disinfectant before reuse.
3. Waste pick up and disposal of Biohazardous Waste
 - a. Sharps waste shall be picked up by the Biosafety Officer, or other designated safety personnel.
 - b. Disposal of autoclaved material is the responsibility of the research lab. All autoclaved solid waste must be taken directly to the dumpsters behind the Science Lab Center.

C. HANDLING MEDICAL WASTE AND ALL HUMAN WASTE

1. All human liquid blood and tissue (infected or not):
 - a. Must be collected in red biohazard bags, and,
 - i. For research labs: Given to the Biosafety Officer for proper disposal,
 - ii. For Health Center or Kinesiology Personnel: Taken directly to the Biohazard Waste barrels at the Storage Room at the Student Health Center.
2. All sharps waste that has been contaminated with infectious agents will be disposed of as Medical Waste at CSUF.
3. Treatment and Shipment Deadlines

Medical waste must be collected and shipped off campus either:

 - a. Within 7 days, if stored at room temperature, or
 - b. Within 90 days, if stored at or below 0° C.
 - c. EXCEPTION: Sealed, full sharps containers must be collected and shipped off campus within 7 days, regardless of storage temperature.

D. NON-INFECTED, NON-INFECTIOUS SPECIMEN WASTE

1. Items Which Require Containment
 - a. All animal carcasses, in part or whole⁵, must be either:
 - i. Placed directly into clear plastic bags (provided by the Biosafety Officer).

NOTE: ONLY animals or animal parts can be put in these bags. NO gloves, paper, plastic, needles, foil, etc. shall be put in with the animals.
2. Items Which Require Special Handling
 - a. Animal blood, and body fluids -- fresh, uninfected, untreated body fluids (USDA Grade, for example) must be disposed of in the laboratory garbage disposal.
 - b. Human or animal urine - flush down the laboratory drain with copious amounts of water; rinse the sink well afterwards.

E. AUTOCLAVES

1. Standard Operating Procedure (SOP) for Operating Autoclaves
 - a. Follow the SOP for proper use of the autoclave which is posted by each autoclave and also provided in Addendum E.
 - b. If anyone requires assistance or training in the operation of the autoclave, contact the Biosafety Officer or the Microbiology laboratory technician.
2. Autoclaving waste: Procedures
 - a. Waste must be in an autoclavable bag⁶.
 - b. Bag must be vented to allow proper steam penetration.
 - c. Bag should have a temperature indicator (e.g., autoclave tape).
 - d. Waste must be autoclaved for at least 45 minutes.
 - e. Temperature must reach and be maintained at 250°F. (121°C.) and pressure must be maintained at 15 lbs for the entire autoclave time.
 - f. Set autoclave cycle for "LIQUIDS."
 - g. Place autoclaved waste directly into dumpsters outside the building.

⁵See Appendix C for Formaldehyde/Preserved Carcasses.

⁶Make sure that these are AUTOCLAVABLE bags and withstand the high temperatures of the autoclave.

F. DISPOSAL SUMMARY OF BIOLOGICAL AGENTS

BIOHAZARDOUS (INFECTIOUS) WASTE

TYPE OF WASTE	CONTAINMENT	AUTOCLAVE TREATMENT	DISPOSAL
Sharps (contaminated syringes, needles, razor blades, scalpels, glass slides, cover slips, Pasteur pipets)	Approved Biohazard sharps container (Obtain from Biosafety Officer)	no	Call RISO for pick up.
Animal carcasses and parts	Red Biohazard bags or 5 gallon buckets (Obtain from RISO)	no	Call RISO for pick up, or arrange storage with Animal Resources.
Animal blood, body fluids	Labeled autoclave container	yes	garbage disposal.
Pathogenic bacteria cultures, tissue cultures, viruses, parasites, biologically produced toxins	Labeled autoclave container	yes	Liquid parts: drain, flushed with copious amounts of water. Solid parts: trash.
Disposable plastics and other trash	Labeled autoclave bag	yes	Trash. If any broken glass or glass pipets present, place bag in cardboard box before disposal in trash.

NON-BIOHAZARDOUS (NON-INFECTIOUS) WASTE

TYPE OF WASTE	CONTAINMENT	DISPOSAL
Sharps	Puncture-proof container	Seal tightly, label and place in trash.
Disposable pipets and other disposable glassware (NO LIQUIDS)	Heavy cardboard boxes, seams sealed with tape to prevent shards from coming out.	Seal tightly with tape, label and place in trash.
All animals carcasses and parts	Clear plastic bags. NO plastic, paper, needles, foil, etc. should be put in with the animals.	Place in refrigerator or freezer, then notify Biosafety Officer to pick up.
Animal blood, body fluids	Labeled container	Flush down drain using garbage disposal, if possible; flush sink with copious amounts of water.

VI. OFF-CAMPUS ACTIVITIES INVOLVING BIOHAZARDS

- A. All CSUF personnel engaged in off-campus activities involving biohazards must abide by the same regulations and guidelines as outlined in this manual. When an off-campus site has stricter regulations than outlined here, the stricter regulations will apply.

VII. EMERGENCY PROCEDURES

Appropriate handling, deactivation and emergency procedures must be posted in all areas where biohazardous work occurs. In all cases, the Principal Investigator or Course Instructor and the Biosafety Officer, or Public Safety must be notified.

A. SPILLS

1. Isolate area to prevent spread of contamination.
2. Follow posted deactivation procedures.
3. Notify other personnel in the immediate area.

B. PERSONNEL CONTAMINATION

1. Remove contaminated clothing.
2. Wash exposed area with soap and rinse with water for at least 5 minutes.
3. Obtain medical attention, if necessary.

KEY CONTACTS:

Name	Room No.	Extension
Biosafety Officer	MH557	2507 or 2687
Radiation Safety Office	MH557	2687
Chemical Hygiene Officer (CHO)	MH557	5938 or 2687
Public Safety (from campus offices)	---	911
Environmental Health & Instructional Safety Office	T-14	7233
Animal Resources	MH231	2807
Student Health Center	HC	2800

VIII. APPENDICES

APPENDIX A. HAZARD HIERARCHY

Biohazards which contain hazardous chemicals or radioactive material require special handling. See Radiation Safety Officer and Chemical Hygiene Officer for the specific use and disposal or treatment requirements of the hazards present.

A. If biohazardous and radioactive:

1. Inactivate biohazard, if possible. (Use procedures approved by the Radiation Safety Officer which restrict further contamination.) Classify as radioactive and treated accordingly.
2. See Radiation Safety manual for treatment of radioactive materials.

B. If chemically hazardous and biohazardous:

1. Classify as chemically hazardous and treat accordingly.
2. Call Chemical Hygiene Officer for specific instructions for that chemical.

C. If biohazardous, chemically hazardous and radioactive:

1. Classify as radioactive and treated accordingly.

D. If chemically hazardous and radioactive (no biohazards present):

1. Contact Radiation Safety Officer for specific treatment for those isotopes and chemicals.

APPENDIX B. NIH GUIDELINES FOR AN INSTITUTIONAL BIOSAFETY COMMITTEE

Published in the Federal Register, Volume 51, No. 88, May 7, 1986, under the Department of Health and Human Services, the "Guidelines for Research Involving Recombinant DNA Molecules" state, in part:

"IV-B---Responsibility of the Institution

IV-B-1. General Information. Each institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the institution shall:

IV-B-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines...

IV-B-1-b. Establish an IBC (Institutional Biosafety Committee) that meets the requirements set forth in Section IV-B-2 and carries out the functions detailed in Section IV-B-3.

IV-B-2. Membership and Procedures of the IBC. The institution shall establish an IBC whose responsibility need not be restricted to recombinant DNA. The committee shall meet the following requirements:

IV-B-2-a. The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment...

IV-B-2-b. In order to ensure the competence necessary to review recombinant DNA activities, it is recommended that: (i) The IBC include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) the IBC include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be from the laboratory technical staff..."

See the Biosafety Officer (MH-557) or Dr. Stuart Ross in the Grants and Contracts Office (MH-112) for the complete text of these Guidelines.

APPENDIX C. FORMALDEHYDE/PRESERVED CARCASSES

Any animal carcass or animal parts which have been preserved in formaldehyde or any other preservative must have as much of the preservatives leached from the carcass as possible and be drained completely of any fluids before they can be placed in any container for disposal. Please note that all work with formaldehyde must be done in a fume hood and with proper personal protection equipment (safety glasses, gloves, lab coat). Reserve all fluids for collection by the Chemical Hygiene Officer. If carcasses also contain infectious/pathogenic agents, contact Research and Instructional Safety Office before disposal.

APPENDIX D. SOLID WASTE DISPOSAL SUMMARY OF LABORATORY WASTE (In trash cans or dumpsters)

NO	YES
1. Liquids of any kind 2. Infected sharps 3. Biohazards 4. Chemicals 5. Radioactive material 6. Loose, broken, sharp-edged glass* 7. Animal carcasses	1. Autoclaved waste (NO liquids) 2. Regular trash 3. Properly-contained broken glass*

*Glass items may be disposed of in the regular trash if they contain no hazardous chemicals, radioactive materials or biohazardous materials. Place items in a heavy duty cardboard box and tape all seams shut to prevent leaking of glass shards. Clearly mark the box "CAUTION--BROKEN GLASS" and leave it with your normal trash, or take directly to outside dumpsters. OR, place glass in the foot-operated glass waste containers in the laboratories. Call the Ext. 2687 for pick up, or with proper protective equipment (lab coat and eye protection) containers may taken to the outside dumpsters.

APPENDIX E. STANDARD OPERATING PROCEDURE FOR OPERATING AUTOCLAVE FOR WASTE

To load:

- a. Normal jacket pressure is 15 pounds. Normal temperature is 250°F.
- b. Select LIQUIDS setting.
- c. Adjust timer to 45 minutes.
- d. Put material into autoclave and close door.

SLC AUTOCLAVES: Press START.

MH AUTOCLAVES: First the inside latch must be locked by turning it to the right, next the outside handle must be tightened by rotating to the right until it is tight. Do not overtighten, but do tighten outside handle firmly. **Turning the handle starts the cycle.**

To unload:

- a. Wait 10-15 minutes after cycles is complete to allow items to cool. Hot liquids could boil over if disturbed before some cooling occurs.
- b. Open door. **REMEMBER: DO NOT STAND IN FRONT OF DOOR AS YOU OPEN IT.**

HOT WATER VAPOR WILL COME OUT!

SLC: Pulling door handle down a couple inches to allow steam to escape. After steam has exhausted, slowly lower door fully.

MH: Unlock door by rotating outside handle to the left. (first movement may be difficult if overtightened). Next unlatch inside handle by turning it to the left. Stand behind door as you swing it open.

- c. Remove items with gloves as they will be hot.
- d. Close autoclave door.

MH ONLY: Latch the inside handle. Remember: **Do not tighten the outer handle** as this will automatically start another round of autoclaving.

IX. REFERENCES

(Available in MH-557)

1. Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Public Health Service, CDC and NIH, 2nd Edition, May 1988.
This guide is available online at: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>
2. Biosafety in the Laboratory, Prudent Practices for the Handling and Disposal of Infectious Materials, National Research Council, National Academy Press, 1990.
3. Finding the Rx for Managing Medical Wastes, Congress of the United States Office of Technology Assessment, 1990.
4. "Medical Waste Management Act," California State Assembly Bills No. 109 and 1641, which adds Chapter 6.1 of Division 20 to the Health and Safety Code and amends various other sections of the Business and Professions code and the Public Resources Code, relating to health. The complete text of the California Medical Waste Management Act (MWMA) is available at the California Department of Health site at:
http://www.dhs.ca.gov/ps/ddwem/environmental/med_waste/medwasteindex.htm