

**Section 3: BIOSAFETY LEVEL 2 LABORATORIES****TABLE OF CONTENTS**

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**A. Codes, Standards, and References**

California Code of Regulations (CCR), Title 8, General Industry Safety Orders, Section 5193, **Bloodborne Pathogens**

California Code of Regulations (CCR), Title 8, General Industry Safety Orders, Section 5154.2, **Ventilation Requirements For Biosafety Cabinets**

California Code of Regulations (CCR), Title 8, General Industry Safety Orders, Section 3203, **The Injury Illness and Prevention Program (IIPP)**

California Health and Safety Code; Part 14, **The California Medical Waste Management Act**

National Fire Protection Association (NFPA) Standard 45, Fire Protection for Laboratories

The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), **Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 2<sup>nd</sup> Edition**

The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), **Biosafety in Microbiological and Biomedical Laboratories, 4<sup>th</sup> Edition**

**NIH Guidelines for Research Involving Recombinant DNA Molecules**, April 2002

***National Sanitation Foundation (NSF) International Standard 49***

**B. Scope**

All of the biological research conducted at Stanford University involves low to moderate risk etiological agents as defined by the NIH. Section 1 of this Guide, General Requirements for Stanford Laboratories, covers all design requirements for Biosafety Level 1 laboratory work areas. This section focuses primarily on the biosafety considerations for a Biosafety Level 2 laboratory.

Proposed Biosafety Level 3 labs will be reviewed on a case by case basis depending on what biohazard material the principal investigator plans to use.

[NOTE: The use of sheep specimens are regulated by a 1979 Special Order from Cal/OSHA, and any request for new laboratory space to utilize such specimens must comply with this special order. Likewise, the use of any specimens of ***Mycobacterium tuberculosis*** is subject to review based on the proposed TB standard, California Code of Regulations (CCR), Title 8, Section 5197.]

**C. Ventilation Considerations for Biosafety Level 2 Laboratories**

- 1. Air pressure in laboratories and animal care rooms should be negative in relation to the corridor or adjacent non-laboratory areas. Rooms housing immunocompromised animals should be at a positive pressure with respect to adjoining areas. Consult with SU Fire Marshall for design details.**

CDC-NIH Biosafety in Microbiological and Biomedical Laboratories (ABSL 2, D.5)  
Good Practice per Stanford EH&S

Potentially harmful aerosols can escape from the containment of the laboratory room unless the room air pressure is negative to adjacent non-laboratory areas. As a general rule, air should flow from low hazard to high hazard areas.

- 2. Dedicated sterile tissue culture rooms should be balanced neutral or slightly positive with respect to adjoining areas. Tissue culture rooms that involve the use of biohazardous agents shall be negative as stated in C-1 above.**

Good Practice per Stanford University EH&S

This will minimize the potential for possible contamination of experiments within these rooms.

- 3. An autoclave should be provided with a canopy hood, slotted exhaust, or other suitable means of local exhaust. In addition, autoclave rooms should have a minimum of 10 air changes per hour.**

Good Practice per Stanford University EH&S

Unpleasant heat and odors will linger in the room unless provided with effective local exhaust and adequate frequency of air changes.

#### **D. Biological Safety Cabinets and Other Containment Considerations**

##### **➤ Approval/Type**

- 1. All cabinets must be NSF listed, UL approved, and installed in accordance with the manufacturer's requirements.**

Good Practice per Stanford University

Cabinets, which when used and installed properly, will provide both product and personnel protection. However, if the cabinet is not installed properly (e.g., not ducting a Class II, B2 cabinet), then it will not be serviceable. Installation of a cabinet which deviates from the listed NSF requirements, will void the NSF Standard 49 approved listing.

- 2. For Biosafety Level 2 applications involving toxic chemicals or radionuclides, a**

**Class II- B type cabinet must be installed.**

Good Practice per Stanford University EH&S

Class II-B cabinets do not allow in-room venting of exhaust air and are thus appropriate for such uses. For Biosafety Level 2 applications, fume hoods are not appropriate; a fume hood is not designed for the usage of biological materials. An appropriate biosafety cabinet must be used. The exact type of BSC should be specified early in the design process.

**➤ Venting**

- 3. The Biosafety cabinet shall be vented from the building if toxic or malodorous chemicals are used. A thimble connection to the exhaust is one way to exhaust a Class IIA cabinet.**

Primary containment for Biohazards, CDC/NIH  
Good Practice per Stanford University EH&S

- 4. Venting to external ducts shall be monitored.**

8 CCR 5142

Where cabinets are connected to external ducts, a flow monitoring system with audible and visual annunciators shall be used to alert the cabinet users of loss of external ventilation. Alternatively, thimble connections or canopy mini-enclosures in cabinets shall be fitted with a ribbon streamer or equivalent attached at an edge through which air enters the device to indicate the airflow direction.

**➤ Location**

- 5. Biological safety cabinets (BSCs) must be located away from doors and other high traffic areas.**

NSF Standard 49, Annex E, I.A.1  
Good Practice per Stanford University EH&S

Currents of air can disrupt and degrade the protective capability of the cabinet. All attempts should be made to neutralize any interferences.

- 6. A biosafety cabinet should not be installed directly opposite of another biosafety cabinet if spatial considerations allow otherwise.**

NSF Standard 49, Appendix E  
Good Practice per Stanford University EH&S

Laminar airflow is greatly hindered by the operation of a biosafety cabinet located directly opposite of another biosafety cabinet or autoclave. It is recommended to provide at least six feet between cabinets.

- 7. A biosafety cabinet should not be installed directly under air supply inlets**

NSF Standard 49, Appendix E  
Good Practice per Stanford University EH&S

External air currents degrade the effectiveness of Biosafety cabinets. If possible, locate cabinets where air supply inlets will not interfere with performance

**8. A Biosafety cabinet should not be installed within ten feet of an autoclave.**

Good Practice per Stanford University EH&S

Exhaust from an autoclave may contain heat and moisture that will blow into the face of the cabinet. This will cause air turbulence in the cabinet and adversely affect the performance of the unit. There is also an increase of potential contamination within the cabinet if the autoclave is not functioning properly since the steam may contain spores or aerosols.

**9. A 12-inch clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance, and to ensure that the air return to the laboratory is not hindered. When the BSC is hard-ducted or connected by thimble unit to the ventilation system adequate space must be provided so as not to interfere with air flow.**

Primary containment for Biohazards, CDC/NIH  
Good Practice per Stanford University EH&S

These placement considerations are required to ensure maximum effectiveness of the primary barrier (BSC).

➤ **Natural Gas**

**10. Open flames are not to be used in Biosafety Cabinets**

Good Practice per Stanford University EH&S

**Stanford University has taken a strong stance against the use of gas burners or alcohol flames in Biosafety cabinets.** The decision has been made in accordance with recommendations from numerous agencies. The Center for Disease Control and Prevention (CDC) reports that 'open-flames are not required in the near microbe-free environment of a biological safety cabinet' and create 'turbulence which disrupts the pattern of air supplied to the work surface' jeopardizing the sterility of the work area. This is also the recommendation of the World Health Organization (WHO) as well as the major Biosafety cabinet manufacturers.

➤ **Restraints**

**11. All biosafety cabinets must be provided with an appropriate means of seismic stabilization.**

Good Practice per Stanford University EH&S

(Note: The manufacturer should always be consulted to avoid possible damage to the pressurized cabinet volumes.)

➤ **Autoclaves**

**6. Laboratory design must include an autoclave for sterilizing media, lab instruments, and medical waste as necessary.**

CDC-NIH Biosafety in Microbiological and Biomedical Laboratories (BSL 2, D.6)  
Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) App. G-II-B-4-f

An autoclave is required since heat and pressure can kill potentially infectious spores that resist other disinfectants. The autoclave need not be in the actual lab room, however should be available in close proximity. All autoclaves shall be seismically anchored. All medical/biohazardous waste must be disposed in a manner consistent with the Stanford Biosafety Manual section on biohazardous waste.

**E. Biohazardous/Medical waste**

**Biohazardous waste must be contained in appropriate secondary containers prior to disposal.**

The California Medical Waste Management Act

Biohazardous and medical waste must be placed in 'red bags' which are located within approved secondary containment. These waste receptacles are in addition to the non-hazardous waste bins used within the laboratory. Sufficient floor space must be planned in order to have enough room for the necessary waste containers.

**F. Additional Considerations for HIV/HBV Research Laboratories**

**1. HIV/HBV research laboratories shall have vacuum lines which are protected with liquid disinfectant traps and high efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency. (Note: Filters must be maintained and routinely replaced, as necessary).**

CCR, Title 8, Section 5193 (e)(2)(B)9

Liquid disinfectant traps and HEPA filtered vacuum lines prevent inadvertent contamination resulting from a release or backflow of liquid HIV/HBV contamination through a laboratory vacuum line.

**2. HIV/HBV research laboratories shall contain a facility for handwashing and an eyewash facility which is readily available within the work area.**

CCR, Title 8, Section 5193 (e)(3)(A)

Containment equipment such as a sink and eyewash will expedite personnel decontamination in the event of a splash or spill on the body. For information on the appropriate eyewashes that meet EH&S approval, review Section 1.2, Emergency Eyewash and Safety Showers in this Guide.