

BSL-3 Laboratory Design (Biosafety Level 3)

REVISED

Environmental Health Program
and
Office of Biological Safety

University of Wisconsin Madison

November 8, 2005

This document describes University of Wisconsin - Madison facility design criteria that are applicable to construction of new campus laboratories intended for BSL-3 activities. Existing facilities should consider upgrading to meet these criteria, and in certain circumstances such as when undergoing remodeling, may be required to do so.

This document is intended as an aid to architects, engineers, building managers, contractors and safety inspectors to facilitate the design, construction, commissioning, and inspections of BSL-3 laboratories.

Acknowledgments:

Darren J. Berger
Jan A. Klein
Jack S. Wunder
Rick A. Johnson

Revision 3 - November 8, 2005

Revision 2 - August 2005

Revision 1 - 1999

January 1994

I. Preliminary	
A. General Introduction to Design.....	1
B. Application of Codes, Regulations, Guidelines at UW-Madison	1
C. Scope	1
D. Definitions	2
E. Table 1 - Summary of Facility Standards	5
II. Basic Laboratory Design Features	
A. Physical Separation	6
B. Interior Surfaces.....	6
C. Bench Tops	6
D. Laboratory Furniture.....	6
E. Hand-washing Sink	7
F. Windows.....	7
G. Laboratory Doors	7
H. Air Ventilation System	7
I. Autoclave	7
J. Autoclave Ventilation.....	7
K. Eyewash Station	8
L. Light Fixtures.....	8
M. Clothes Change Area.....	8
III. Ventilation Features	
A. Supply Air.....	8
B. Exhaust Air.....	9
C. Directional Air Flow	10
D. Controls/Interlock	11
E. Sound Control	11
IV. Safety Equipment	
A. Biological Safety Cabinets.....	11
B. Restricted Ventilation Devices.....	13
C. Animal Housing/Bedding/Transfer Stations.....	14
D. Centrifuges.....	14
E. Vacuum Lines	14
F. Compressed Gas Cylinders	14
G. Natural Gas in Biological Safety Cabinets.....	15
V. Architectural Considerations	
A. Aisle Clearance	15
B. Electrical Outlets	15
C. Room Temperature and Humidity	15
D. Laboratory Benches	16
E. Circuit Breakers.....	16

F. Gas and Vacuum Shut-off Valves	16
G. Union and Gas Shut-offs	16
H. Chemical Storage.....	16
I. Security	17
VI. Certification, Maintenance, and Commissioning	
A. Biological Safety Cabinet Certification.....	17
B. Decontamination of Biological Safety Cabinets.....	17
C. Autoclaves.....	17
D. Centrifuges.....	17
E. Annual Testing of Exhaust Air Filtration Systems.....	17
F. Negative Room Pressure	18
G. Laboratory Inspections	18
H. Commissioning.....	18
Appendix A	
Regulations	20
Guidelines	21
Standards.....	22
Appendix B	
Clean Air and Biological Safety Cabinet Items on UW Contract (05-5770)	23
Appendix C	
Testing and Certification of BSL-3 and BSL3-Ag Containment Rooms.....	24
Appendix D	
BSL-3 Manual - Points to Consider.....	27

I. Preliminary

A. General Introduction to Design

Laboratories should be designed to address the hazards that are inherent or anticipated in such facilities. The design team must consider the following major hazards that are encountered in biomedical laboratories:

1. **Biological:** infectious agents, exotic organisms, clinical and other materials that may contain pathogens and/or toxins of biological origin.
2. **Chemical:** carcinogens, mutagens, teratogens, toxic chemicals, anesthetic gases, flammables, compressed gases, etc.
3. **Physical:** lasers, magnetic fields, high voltage, ultraviolet light, high noise levels, electromagnetic fields, vibration.
4. **Radioactive:** radionuclides, equipment that produces ionizing radiation.

The methods of handling these hazards in the laboratory are critical to the laboratory design. Therefore, the risk of each hazard must be individually assessed during the design process, incorporating appropriate measures to provide for proper storage, handling and disposal of the identified hazards. Designing for the ease of usage must consider the research activities that will be conducted in the lab space as part of the process. Regulations, guidelines and standards developed to address these concerns are referenced throughout this document. All references are listed in Appendix A.

B. Application of Codes, Regulations, Guidelines at UW-Madison

Regulations: requirements according to rules adopted by a governmental agency under legal authority.

Guidelines: recommendations detailing best practice, which are adopted to pertain to a given situation.

Policy: standards published by recognized national organizations (e.g., National Fire Protection Agency (NFPA), American Biological Safety Association (ABSA), National Sanitation Foundation (NSF), Office of Biological Safety (OBS), Institutional Biosafety Committee (IBC)) as being required or best practice and preferred for UW-Madison facilities. Standards may be incorporated into governmental codes.

C. Scope

Biosafety Level 3 (BSL-3) laboratories are those in which work is done with infectious microorganisms, indigenous or exotic, considered by NIH/CDC to be risk group 3 pathogens. These agents are capable of causing serious or potentially fatal disease as a result of exposure, usually by the inhalation route. Slightly more specialized and restrictive is the BSL3-Ag facility, required by USDA-APHIS for high consequence animal pathogens which could have significant impact on the agricultural sector of the economy.

The BSL-3 containment facility is designed with special engineering and facility features. Biological safety cabinets are an essential engineering control of BSL-3 laboratories and are required for most procedures involving manipulations of infectious materials. Because research with risk group 3 pathogens sometimes involves the other major hazard classes, all aspects of safety must be coordinated in the design of the laboratory.

This design guide will focus on the **biosafety considerations** for a BSL-3 laboratory. If radionuclides are used, specific guidelines for safe containment may be obtained from the Radiation Safety Officer (262-8769). If volatile solvents, corrosive chemicals or any of the physical hazards are involved, safety guidelines and relevant regulations for safe containment may be obtained from the Chemical Safety and General Safety groups within the University of Wisconsin-Madison Safety Department (262-8769) or the University Health Service Environmental Health Program (262-1809).

D. Definitions

Aerosol: a suspension in air of liquid or solid particles typically less than five microns in diameter. If inhaled, such particles can infiltrate the alveoli of the lungs.

Biological Safety Cabinet (BSC): a ventilated enclosure for personal, product and environmental protection. It is characterized by a protective laminar flow air barrier and HEPA-filtered supply and exhaust air.

Class II BSC: These BSCs are available in several types that vary by the ratio of air exhausted versus re-circulated and whether the contaminated plenum is under positive or negative pressure. Some are installed with a canopy exhaust connection; others may be hard ducted to the outside. The configuration must be carefully considered when selecting the appropriate BSC for use with hazardous chemical vapors and maintaining air balance in the room.

Class III BSC: A glove box or closed front ventilated enclosure with negative pressure and gas tight construction providing total protection for personnel and product from contaminants exterior to the cabinet.

Canopy: an exhaust enclosure used to capture air containing heat, moisture or nuisance odors. Typical application includes: autoclaves, washing stations, kitchens, etc.

Canopy exhaust connection: a sheet metal transition piece located above the exhaust from a Class II, A1 or A2 biological safety cabinet. It does not alter the balance of the BSC exhaust system by providing a small air gap (usually 1 inch) around the BSC exhaust filter housing. The volume of the room exhaust must be sufficient to maintain the flow of room air and that of the BSC. Check the manufacturers specifications for this minimum exhaust volume.

CAD: a clean air device or laminar flow clean bench is an enclosure that provides HEPA filtered supply air to the work surface - product protection only. Since there is no personal protection, do not use this equipment with hazardous or potentially hazardous materials or animals.

CFM: a unit of volumetric measurement over time, cubic feet per minute.

CFR: Code of Federal Regulations

Commissioning: The process of ensuring that systems are designed, installed, functionally tested, and capable of being operated and maintained to conformity with the design intent.

Containment: a method of safely managing infectious agents within the laboratory environment to prevent their escape from the laboratory.

Db(A): Measure of sound that is weighted to simulate actual human auditory function.

Decontamination: a procedure that eliminates or reduces microbial contamination (or toxic substances) to a safe level with respect to transmission of infection (or toxicity).

Disinfection: a treatment to destroy harmful microorganisms.

EHP: Environmental Health Program (262-1809), the support team that certifies and maintains BSCs for the UW-Madison.

FPM: a unit of velocity measurement, feet per minute.

HEPA Filter: High efficiency particulate air filter captures airborne particulates, including microorganisms. Each HEPA filter must trap at least 9,997 to 9,999 of every 10,000 particulates which are 0.3 micron in diameter. The HEPA filter traps particulates greater or less than 0.3 microns with greater efficiency. Particulates of 0.3 microns are the most penetrating particle size. The UW specifies that 99.99% HEPA filters are the minimum standard for hazardous exhaust applications.

IBC: Institutional Biosafety Committee—a committee delegated with the responsibility for the safe and compliant conduct of all potentially biohazardous research at UW-Madison.

IVC: Individually ventilated cage for animals.

Metasys: a building automation system used to manage and communicate equipment performance.

OBS: Office of Biological Safety (263-2037), the support team that provides biosafety guidance to UW-Madison and the administrative office of the IBC.

PPE: Personal protective equipment (e.g., gloves, lab coat, eye protection, booties, face mask, respirator, hearing protection).

Primary Barrier: Specialized equipment that is designed for capture or containment of biological agents. Biological Safety Cabinets and animal cage dump stations are examples of larger primary barriers. Sealed centrifuge cups, bioaerosol centrifuges, aerosol-containing blenders or high speed mixers, and related devices are examples of smaller primary barriers.

Noise or Room Criteria (NC or RC 45): Level of noise in a room that allows personnel to talk normally without interruption.

Risk Group (RG): In the U.S., the most current classification is found in the NIH Guidelines for Research Activities Involving Recombinant DNA Molecules. The human pathogens 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 high hazard infectious agents. In general, the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures and/or effective treatment are some of the criteria taken into consideration when classifying infectious agents.

Relative Humidity (RH): the local humidity of the room based on standard temperature and pressure.

Secondary Barrier: These are facility-related design features and operational practices that protect the environment external to the laboratory from exposure to biohazardous materials (from one interior area to another, or from the interior of the facility to the outside environment). Examples of secondary barriers include work areas that are separate from public areas, decontamination and hand washing facilities, special ventilation systems, airlocks, directional airflow through the use of air pressure differentials, double door autoclaves opening to the exterior, air gasketed doors (interior and exterior). All personnel practices that are involved in maintaining these systems, or in minimizing personal contamination and the spread of infectious microorganisms, are also an integral part of the secondary barrier system, as is good laboratory housekeeping.

Inches of Water Column (" WC): a medium (water) used to measure air pressure, usually inches of water column (" WC).

Table 1
Summary of Facility Standards Recommended for Biosafety Levels

	Biosafety Level			
	1	2	3	3-Ag
Laboratory visit by OBS	Desirable	Desirable	Yes	Yes
Isolation of laboratory from public areas	---	---	Desirable	Yes
Eye wash, plumbed	Desirable	Yes	Yes	Yes
Interior surfaces (impervious, cleanable):	Yes	Yes	Yes	Yes
Bench tops	Yes	Yes	Yes	Yes
Laboratory furniture	Yes	Yes	Yes	Yes
Floors - conventional (no carpet)	Yes	Yes	---	---
Floors - seamless, integral cove base	---	Desirable	Yes	Yes
Ceiling - conventional	Yes	Yes	---	---
Ceiling - permanent, fixed	---	---	Yes	Yes
Sinks in laboratory	Yes	Yes	Yes	Yes
Hands-free	---	Desirable	Yes	Yes
Water supply protected	---	---	^a Yes	^a Yes
Windows allowed	Yes	Yes	Yes	Yes
May be opened	^a No	^a No	No	No
Must be sealed	No	No	Yes	Yes
Room penetrations sealed for gas decontamination (pressure decay testing)	No	No	Desirable	Yes
Ventilation (single pass supply/exhaust)	Yes	Yes	Yes	Yes
Inward air flow (neg. pressure)	Yes ^a	Yes ^a	Yes	Yes
Mechanical via centralized system	Yes	Yes	Yes	No
Mechanical, independent system	No	No	Desirable	Yes
Filtered exhaust required	No	No	Desirable	Yes
Interlocked supply required	No	No	Yes	Yes
Annually test filters/HVAC systems	No	No	Yes	Yes
Annually test controls/alarms	No	No	Yes	Yes
Doors (self closing):	Desirable	Desirable	Yes	Yes
Double-door entry required	No	No	Yes	Yes
Airlock (anteroom)	No	No	Desirable	Yes
Autoclave on site	Desirable	Yes	Yes	Yes
In laboratory room	---	---	Desirable	Yes
Pass thru (double-ended)	---	---	Desirable	Yes
Biological Safety Cabinets				
Annual Certification	Desirable	Yes	Yes	Yes
Class I or Class II	---	Desirable	Yes	Yes
Class III	---	---	Desirable	Yes
Vacuum Lines should be protected liquid trap and in-line HEPA filter	Desirable	Yes	Yes	Yes
Shower facility in airlock	---	---	Desirable	Yes
Waste Effluent Treatment System	---	---	Desirable	Yes
Centrifuge with sealed rotors	---	Desirable	Yes	Yes

^a Required by Wisconsin Administrative Code.

--- Not Applicable/Needed

Existing facilities that do not meet these recommendations should plan to address deficiencies during future maintenance or remodeling. Contact OBS for assistance.

II. Basic Laboratory Design Features

- A. **Physical separation:** The BSL-3 laboratory is separated from areas that are open to unrestricted traffic within the building. Passage through two self closing lockable doors is the basic requirement for entry into the BSL-3 laboratory from common corridors or other contiguous areas. A clothing change room with or without showers may be included in the passageway within the airlock.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.1; NIH Guidelines; USDA

- B. **Interior surfaces:** The interior surfaces of the BSL-3 laboratory walls, floors and ceilings must be water resistant and easily cleanable. **Sealed penetrations** in these surfaces are an integral part of the ventilation system, which is designed to maintain room negative air pressure and to facilitate decontamination. Pressure testing is recommended to verify tight construction particularly if designed to be gas decontaminated. The mode of gas decontamination must be effective and compatible with materials and equipment used for research. The walls are painted and sealed in a manner that facilitates washing for routine decontamination and in the event of a splash. Acoustical and detachable lay-in perforated ceilings are unacceptable. Wood-finished walls or floors are problematic because this surface is difficult to maintain and can absorb potentially infectious material. Monolithic floors are required: a continuous floor system or seamless sheet vinyl is preferred although a well-maintained tile floor meets the standard. A continuous floor reduces the potential of liquid absorption. Integral covings are recommended to facilitate clean-up and these should be sealed to the cabinetry and walls. Floorings that allow liquid to seep through the small cracks or gaps (e.g., unwaxed or poorly maintained tile floors) are unacceptable. All rug treatments are prohibited.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.3

- C. **Bench tops:** Lab bench tops must be impervious to water and resistant to acids, alkalis, organic solvents and moderate heat. BSL-3 manipulations can involve concurrent use of chemical solvents such as formaldehyde, phenol and ethanol as well as corrosives. The laboratory bench must be resistant to the chemical actions of these substances and disinfectants in gas or liquid state. Wooden bench tops are not appropriate because a wood surface mars easily and such that it cannot readily be decontaminated. Also wood burns rapidly in the event of a fire. Fiberglass is inappropriate since it can degrade when strong disinfectants are applied and it releases toxic smoke when burned.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.4

- D. **Laboratory furniture:** Laboratory furniture must be sturdy, should have impervious surfaces to resist the absorption of liquids and the harsh effects of disinfectants. Wood cabinetry is problematic to maintain in a condition that meets the requirement to be impervious and easily decontaminated. Metal furniture should be rust resistant and tolerant of corrosive disinfectants. Spaces between benches, cabinets and equipment should be accessible for cleaning or eliminated. Furniture must be positioned in a manner that makes it easy to mitigate spilled liquids or conduct routine maintenance. A minimum distance of 18 inches above the BSC must be unobstructed due to limited space that might

not allow the biosafety cabinet certifier to remove panels of the cabinet when recertifying the unit. Shelving above a BSC within an 18-inch vertical distance from the exhaust filter housing interferes with filter maintenance and increases turbulence.

Reference: CDC/NIH BMBL, BSL-2, Section III.D.5

- E. **Hand-washing sink:** Certain pathogenic organisms can be transferred by hand contact to mucous membranes or to other surfaces in the laboratory. A sink for hand washing that operates hands-free (foot pedal, elbow levers, or automatic) is required in the BSL-3 laboratory. It should be located near the laboratory exit door. Another small sink in the anteroom allows hands to be washed again prior to exiting the BSL-3 facility.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.2

- F. **Windows:** To maintain air balance and prevent egress of aerosols to the surrounding environment, windows in the laboratory must be fixed and permanently sealed once the facility is designated as BSL-3.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.6

- G. **Laboratory doors:** Access doors to the laboratory or containment module are to be self-closing. BSL-3 laboratories are mechanically ventilated spaces. Doors left open are a breach in containment and will allow the escape of contamination. Doors need to be kept closed when not in use. Pocket doors are not appropriate because the recessed design makes cleaning difficult.

Reference: CDC/NIH, BSL-3, Section III.D.1

- H. **Air ventilation system:** To prevent the migration of airborne contaminants, a ducted exhaust ventilation system must be provided (see Section III below). The BSL-3 laboratory is an independently, mechanically ventilated space that must be physically separated from other spaces.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.9; NFPA 45, Section 6.4.2

- I. **Autoclave:** An autoclave should be available within the laboratory for disinfection of contaminated materials before they are removed from the containment suite. On occasion, due to certain circumstances (e.g., space limitation), this requirement may be waived if an autoclave is located nearby. The autoclave must be within the BSL-3 facility if two or more BSL-3 laboratories share a common anteroom.

Reference: CDC/NIH BMBL BSL-3, Section III.D.7

- J. **Autoclave ventilation:** Autoclaves commonly release hot moist air when opened after being run. This exhaust should be vented via a canopy installed above the door and may require HEPA filtration. The canopy will control humidity, malodors, heat gain and potential contamination of other areas exterior to the BSL-3 envelope.

Reference: DSF Laboratory Design Guideline

- K. **Eyewash station:** Install a plumbed eyewash station in the BSL-3 laboratory that is capable of delivering not less than 0.4 gallons per minute of tepid ($70^{\circ}\text{F} \pm 5^{\circ}\text{F}$), potable water to both eyes for 15 minutes. The nozzle should be protected from airborne contaminants and the valve designed so the water flow remains on without use of the operator's hands. Many pathogens can be infectious via the mucous membrane route. Therefore it is important to flush eyes thoroughly after accidental exposure. Unplumbed eyewash stations are prone to contamination and are discouraged.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.13; Standard: ANSI Z358.1

- L. **Light fixtures:** The laboratory lighting fixtures are gasketed or otherwise sealed to maintain the secondary containment barrier and to facilitate cleaning and gas decontamination if required. All service penetrations must be sealed to prevent migration of gaseous formaldehyde or other decontamination agents into other building spaces. The lab should be verified as sealed before being put into operation.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.3

- M. **Clothes change area:** A clothes change area in the entryway is strongly recommended. Plumbed shower stalls are optional but may be required for work with certain pathogens.

Reference: CDC/NIH BMBL, BSL-3, Section III.D.1; USDA

III. Ventilation Features

A ducted ventilation system which creates directional airflow drawing air from "clean" areas (e.g., corridors) into the "contaminated area" (e.g., laboratory or animal room) is essential in a BSL-3 facility. Potentially hazardous bioaerosols will not escape the containment laboratory because the air pressure is negative to the adjacent non-laboratory areas. The exhaust air is not re-circulated to any other areas of the building and is discharged to the outside away from occupied areas and air intakes.

Guideline: CDC/NIH BMBL, BSL-3, Section III.D.9

The essential features of a mechanical ventilation system are:

A. Supply Air

Supply air is introduced to maintain temperature (68-70 degrees Fahrenheit), control relative humidity (30-55%), control particulate levels and maintain the velocity of the laboratory air at less than 25 feet per minute, five feet from the biological safety cabinet(s). If chilled water is not available year round, an independent/supplemental cooling system may be needed to handle the heat loads.

1. Supply Air Filters

Design new supply air handlers with a pre-filter and secondary air filter. The secondary filter is highly recommended to be rated at 95% efficiency. Existing supply air handlers are recommended to have filtration of at least 40% efficiency. Control of particulates into a BSL-3 area reduces the exhaust system maintenance and laboratory cleaning. It serves as an adjunct to research quality by reducing the probability of spurious contamination of research products.

Standard: ASHRAE 52-76

2. Supply Air Diffusers

Design supply air diffusers, such as perforated, round or quadrant sectional, to provide no more than 25 feet per minute terminal throw velocity at five feet from any biological safety cabinet work zone and from the top of the cabinet, while maintaining uniform space temperature and not compromising the cabinet work zone efficacy. Excessive supply air velocity can severely jeopardize the operation of biological safety cabinet air barriers. High air velocity is also implicated in the migration of airborne contaminants.

Guideline: CDC/NIH-BMBL, Appendix A

3. Air Handling Unit — Supply Air

Provide a separate air handling unit for BSL-3 facilities in new laboratory buildings. In existing buildings, a separate air handler is desirable, although an existing air handling system can be modified, provided the modifications comply with interlock control requirements. Separate handling of supply air provides greater control of over-pressurization of laboratory atmosphere if the exhaust fan should fail. Control of supply air in an existing building via dampers that are normally closed has been shown to work, but additional annual testing is required to verify the integrity of the damper controls.

Guideline: CDC/NIH BMBL, BSL-3, Section III.D.9

B. Exhaust Air

The BSL-3 exhaust system is to be designed for maximum safety for laboratory users, building service personnel, local air intakes and roof-top workers. HEPA filtration of exhaust air from BSL-3 laboratories is not routinely required. However, based on a risk assessment for specific conditions, the OBS/IBC may determine that exhaust air from BSL-3 laboratories should be HEPA-filtered before discharge.

1. Biological Safety Cabinets

- a. Vented, hard-connected biological safety cabinets

If Class II, Type B (B1, B2) cabinets are to be installed in the laboratory, air can be exhausted through the cabinets to provide HEPA filtration.

Caution: When a biosafety cabinet is part of the room exhaust system, the exterior ventilation must remain on at all times (24 hours) to maintain the negative room pressure.

- b. Re-circulating, canopy exhaust-connected biological safety cabinets, Class II Type A1 and A2.

If room air is not directly vented through Class II, Type B biological safety cabinets, the laboratory air needs to be mechanically vented. The laboratory venting must be restricted to commercial and/or approved canopy exhaust connections at the discharge filter of each Class II, Type A2 cabinet. Avoid hard duct connections to Class II Type A2 cabinets.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.D.10 and Appendix A

2. HEPA Exhaust Filtration (Not Mandatory)

After review of BSL-3 activities and consideration of the surrounding area, the OBS/IBC may determine that a HEPA-filtered exhaust system is recommended. Whenever possible "Bag In – Bag Out" HEPA and prefilter change out systems are preferred to help minimize service personnel and environmental exposures during filter changes. The system may be located within the laboratory space or in a secure mechanical equipment room. Locate filter caisson no more than eight feet from the floor. To facilitate annual certification and maintenance it is essential to provide caissons with signage, service logs, isolation dampers, sample ports, differential pressure gauges, and prefilters. All filter housings need to be ceiling accessible and must pass a probe test of .001% (ANSI 510).

Guideline: CDC/NIH, BMBL, BSL-3, Section III.D.16

3. Laboratory Exhaust

BSL-3 laboratory duct work shall be pressure tested to 5 CFM at a design static pressure of 2.5 inches water column (" WC), and can be vented through sealed duct work to a roof-mounted fan and a ten-foot, self-supported stack head. Provisions must be made for sealing the duct work and fan for gas decontamination. Based on hazard risk assessment, the BSCs may be connected to the laboratory exhaust system.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.D.9 and D.10

C. Directional Air Flow

The ventilation system for a BSL-3 laboratory provides continuous air flow into the laboratory and controls negative room pressures between 0.01 and 0.05 " WC relative to common corridor. This creates a secondary barrier to prevent release of infectious aerosols and alerts laboratory personnel to mechanical problems. Located mechanical gauges that monitor air pressure (such as Dwyer model 2000-00-N, 3000-00) at the laboratory's exterior entrance. Signage must be provided that identifies the positive and negative sides of the gauge along with the room number serviced by each gauge and detailed instructions.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.D.9

D. Controls/Interlock

An interlock shall be provided to shut down the supply air in the event of an exhaust air system malfunction. An interlock is necessary to prevent pressurization of the

laboratory space if there is mechanical failure of the exhaust fan(s). Pressurization would force contaminated air out of the laboratory and into other building spaces. The interlock is activated via pressure differential between the exterior (clean corridor) and interior (BSL-3 laboratory). If supply air is shut off via the automatic closing of a damper, the location of the damper needs to be indicated with signage. An audible (80 dba) and visual alarm is required; it alerts laboratory personnel of a malfunction. This audible alarm should have a silence feature that resets itself when the problem clears. Exhaust air shut-down during supply malfunction is not necessary or desirable. Exhaust and supply fans should be connected to a building automation system (Metasys). The system should monitor and report fan status, exhaust duct differential pressure, room temperature and room differential pressure. These alarms should connect to UW Police & Security who contacts individuals on call lists: Steamfitter Shop, Building Manager, Principal Investigator or Lab Manager and Biosafety (if assistance is needed). Emergency directions are to be posted at the alarm and included in user's standard operating procedures.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.D.9

E. Sound Control

The sound control within the laboratory space shall be maintained below room criteria noise level of RC 45. Sound above the level will interfere with voice communication and increase the risk of accidents within the laboratory.

Guideline: ASHRAE Hand Book-Applications-1995, Chapter 43 Sound & Vibration Control, Page 43.5 Table 2, Design Guidelines for HVAC System Noise in Occupied Spaces-Research Laboratories with speech communication 40-50 RC.

IV. Safety Equipment

A. Biological Safety Cabinets

Biological safety cabinets (BSCs) are required equipment in BSL-3 laboratories and are considered to be the most important primary containment barrier in such laboratories.

1. Biological safety cabinets, whether purchased through the UW Purchasing Department contract or as part of a building contract, shall meet all purchasing specifications. Extensive testing has shown that purchase of BSCs not meeting required University of Wisconsin specifications results in unsatisfactory units that are not appropriate for BSL-3 areas.

Guideline: UW-Madison Contract 5770

2. The type of cabinet to be used will be determined during the design process by research applicability and the necessity of functioning as part of the laboratory exhaust system. Not all BSCs are appropriate for a specific intended use. The OBS or EHP must be consulted **before** procurement.

Guideline: CDC/NIH BMBL, Appendix A

3. The effectiveness of BSCs is compromised by outside air currents and the movement of laboratory personnel. BSCs must therefore be located away from the laboratory door and other high traffic areas.

Guideline: CDC/NIH BMBL, Appendix A

4. Two BSCs must not be installed closer than 48 inches front to front because laminar air flow is interrupted by concurrent operation of two cabinets within 48 inches of each other. The potential for air turbulence increases when two cabinet operators are working at the same time in the same BSC.

Standard: NSF 49-2002

5. The cabinet manufacturer has designed BSCs that, when used and installed properly, will provide product, environmental and personal protection. However, if not installed properly (e.g., when Class II, B2 is not hard ducted) it may not be serviceable. All cabinets shall be NSF listed, UL approved and installed according to manufacturer and university requirements. Furthermore, to install a cabinet and deviate from the listed NSF requirements will void the NSF 49 approved listing and jeopardize field certification.

Guideline: CDC/NIH BMBL, Appendix A

6. Do not install a biosafety cabinet directly opposite an autoclave, due to the exhaust from an autoclave containing heat and moisture that may interfere with the air barrier of the BSC. This could cause air turbulence in the BSC and adversely affect the unit's performance. 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. Furthermore, operator usage of an autoclave will create traffic in the vicinity of the cabinet air barrier, detrimentally affecting performance.

Guideline: CDC/NIH BMBL, Appendix A

7. HEPA filters in a BSC do not provide protection from chemical vapors, gases or volatile radionuclides. Activities involving the use of minute quantities of toxic chemicals or trace amounts of radionuclides must be performed in a Class II, Type B1, B2 or canopy hood connected A2 cabinet that has been properly vented and field-certified. From a maintenance, testing, and ducting standpoint, a Class II Type B1 is the preferred BSC for these types of applications.

Guideline: CDC/NIH BMBL, Appendix A

8. HEPA filters and gaskets inside a BSC can shift during transport and deteriorate over time, potentially allowing hazardous materials to escape from the cabinet. All BSCs shall be field certified when first installed in the laboratory and at least annually thereafter. They must also be recertified after relocation and most internal repairs. The BSC must be tested annually to assure containment, as well as product and environmental protection.

Guideline: CDC/NIH BMBL, Appendix A; NSF 49, 2002

B. Restricted Ventilation Devices

1. Clean Air Devices

Clean air devices (horizontal laminar flow clean benches) are not permitted in the BSL-3 laboratory. These devices direct air over the experimental product and into the operator's breathing zone, exposing the laboratorian and surrounding area to potentially infectious, toxic, radioactive and sensitizing materials.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.C.4 and Appendix A.

2. Chemical Fume Hoods

a. Ducted chemical fume hoods

A fume hood does not provide sterility for the experimental product, nor does it provide environmental protection. Activities with infectious materials therefore must not be performed in a fume hood. An approved biosafety cabinet must be used to provide proper product, environmental and personnel protection. Fume hoods and their associated make-up air requirements can degrade BSC air barriers and should not be used in the BSL-3 laboratory. Contact OBS (263-2037) or EHP (262-1809) for more information.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.C.4; UW-Madison, Fume Hood Policy & SOP-2001

b. Ductless (re-circulating) chemical fume hoods

Ductless chemical fume hoods filter exhaust air through chemical entrapment filters. These devices are problematic for numerous reasons, including that they often do not provide adequate protection against the variety of chemicals used, their capacity is limited such that filters can load and become ineffective without warning, they often do not provide an adequate face velocity, and they are expensive for the user to maintain. Their purchase is strongly discouraged and will not be approved except in rare circumstances.

Guideline: UW-Madison, Fume Hood Policy & SOP-2001

C. Animal Housing/Bedding/Transfer Stations

1. A BSC should be available for use during aerosol-generating procedures that involve infectious materials and animals. If use of a BSC is not feasible, an appropriate respiratory protection program must be in place.
2. A Class II, Type B (hard ducted to building exhaust system) or Class III BSC is appropriate for procedures that involve hazardous chemicals such as carcinogens, mutagens, or teratogens and animals. If use of a BSC is not feasible, an appropriate respiratory protection program must be in place.
3. Individually ventilated cage (IVC) systems for small animals species provide added protection for the animals as well as the staff present in the animal room.
4. Bedding dumping stations are similar in design to a Class I BSC, so they provide protection to the worker and the environment.
5. Animal transfer stations are used to transfer small animal species from dirty to clean cages on a weekly or biweekly basis. Depending on the research, the animal and bedding may pose a respiratory hazard to the staff. Selecting a transfer station that provides worker and animal protection is very important. Clean air devices do not provide worker protection and are not appropriate for use when there are potential biological (including animal allergens), chemical or radioactive hazards.

Guideline: CDC/NIH BMBL, BSL-3, Section IV and Guide for the Care and Use of Laboratory Animals.

D. Centrifuges:

The use of centrifuges can produce bioaerosols and may result in a breach of containment and laboratory-acquired infections. Centrifuges in BSL-3 laboratories must be equipped with sealed rotor heads or used with sealed safety containers. Continuous flow centrifuges and other equipment that may produce aerosols must be contained within devices that exhaust air through certified HEPA filters.

Guideline: CDC/NIH BMBL, BSL-3, Section III.D.11

E. Vacuum lines:

Vacuum lines must be protected from contamination with liquid disinfectant traps and in-line HEPA filters. These features protect laboratorians and university personnel who service ancillary support equipment.

Guideline: CDC/NIH, BMBL, BSL-3, Section III.D.12

F. Compressed gas cylinders:

Compressed gas cylinders must be secured and located outside the BSL-3 lab to prevent explosion hazards and reduce risk of contamination to outside staff and vendors.

Standards: NFPA 45 and NFPA 99

G. Natural gas connection:

Natural gas use in a BSC is strongly discouraged but it may be used to support controlled combustion by using small-flame sterilizers such as "Touch-o-Matic" burners. When used to support small, controlled combustion, natural gas is not directly released into the cabinet atmosphere; it is not considered a flammable substrate and therefore does not conflict with the prohibition against use of flammable material within cabinets. Use of pre-sterilized supplies and a no-flame sterilizer such as "Bacticinerator" is preferred.

The cabinet must be hard-plumbed with an exterior, accessible gas shut-off valve, union and recommended stainless steel flex connection (such as manufactured by Swaglock). This assembly is connected to a side wall bulkhead connector and natural gas petcock as provided by the cabinet manufacturer.

Use of unauthorized burners or natural gas introduced by soft plumbing through the working face or any other portal is a fire hazard and is prohibited. Such use will be reported to the laboratory supervisor and unauthorized burners or plumbing will be removed.

Standards: NFPA 45 and NFPA 99

V. Architectural Considerations

A. Aisle clearance: The laboratory must have an aisle clearance of at least 36 inches. The main emergency egress shall have a minimum clearance of 36 inches to facilitate rapid departure in case of emergency.

Standard: NFPA 45

B. Electrical outlets:

1. Sealed electrical services prevent the ingress or egress of air via plugged conduits.
2. Electrical outlets must be able to accommodate the current requirements of the equipment used. A 20-ampere dedicated electrical outlet is needed for each BSC. This outlet must be accessible from the top of the BSC without moving it. All BSCs must be electrically tested annually and provided with enough electrical capacity to service interior outlets.
3. The laboratory may have several items of equipment that require large amounts of electrical current. Such items include freezers, biosafety cabinets, centrifuges, and incubators. The room design must take electrical demand into consideration to avoid potential power failure.

Guideline: NEC 2002; NSF 49, 2002

C. Room temperature, humidity and ventilation:

1. The laboratory must be made thermally comfortable (68° F to 70°F and 30-50 percent RH) and maintained under specified negative air pressure before permanent occupancy. The room temperature, humidity, and ventilation are mechanically controlled via an exterior support ventilation system. Appliances often exhaust heat into a room (e.g., REVCO freezer,

incubator, autoclave), and failure to take this effect into consideration may result in an uncomfortably warm work environment.

2. The room HVAC control system must be located external to the room such that service can be performed without entering the BSL-3 spaces with control diagrams posted in an accessible location. It is recommended that the controls follow a standard format such that identical controls, monitors, and alarms are utilized for all campus BSL-3 spaces. An alarm should be tied into an all campus building automation system (Metasys) so that a failure in temperature, humidity, or air pressure is reported to a central terminal for prompt attention.
3. Windows are sealed and never opened for a cooling effect since this will alter the room air balance and breach containment. Cooling with local fans or heating with portable heaters (a fire hazard) are prohibited.

Guideline: ASHRAE; CDC/NIH BMBL, Appendix A

- D. Laboratory benches, equipment, chairs, stools, etc.:** Laboratory benches must not block emergency access to an egress. Laboratory benches, equipment, chairs, stools, and the like must be placed at least 44 inches from an exit.

Guideline: CDC/NIH BMBL, BSL-3, Section III.D.5; NFPA

- E. Circuit breakers:** In the event of an emergency, the BSL-3 laboratory may be unsafe to enter. Therefore, labeled circuit breakers for key electrical appliances must be located outside the laboratory.

Guideline: CDC/NIH BMBL, BSL-3, Section III; NEC 2002

- F. Gas and vacuum shut-off valves:** In the event of an emergency, the laboratory may be unsafe to enter. The shut-off valves for gas and vacuum lines must be located outside the laboratory, easily accessible, and labeled.

Guideline: CDC/NIH BMBL, BSL-3, Section III; NFPA

- G. Unions and gas shut-offs:** Tornados, mechanical vibration, seismic activity, and routine maintenance may cause gas connections to the biosafety cabinet to leak or entirely break off. Release of natural gas in a laboratory is a definite fire hazard, and a properly installed flexible connection minimizes this hazard. As a result flexible connections, unions, and gas shut-off must be used for natural gas connections to the biosafety cabinet.

Guideline: NFPA; NSF

- H. Chemical Storage:** Minimum chemical storage reduces the hazard impact of chemicals coming into contact with an accidental fire. Only the chemicals needed for one week's activity should be stored in the BSL-3 laboratory. Chemicals must be placed in a chemical storage cabinet when not in use.

Guideline: University of Wisconsin Safety Department, Chemical Safety and Disposal Guide

- I. **Security:** Access control is an important component of a BSL-3 laboratory. Proper assessment is essential during the design process of a facility of this type. Contact UW-Campus Police Department for specific guidance.

Guideline: CDC/NIH BMBL, BSL-3, Section III.A.1; UW-Madison, Biosecurity Task Force

VI. Certification, Maintenance, and Commissioning

- A. **Biological safety cabinets certification:** To ensure containment for the protection of laboratory personnel from laboratory-acquired infections and to provide product and environmental protection all biological safety cabinets in BSL-3 laboratories must be certified initially before use, annually thereafter, after moving, and after filter replacement.

Guideline: CDC/NIH BMBL, Appendix A

- B. **Decontamination of biological safety cabinets:** Decontamination protects certification personnel and all individuals involved in disposal of contaminated filters. All BSCs must be decontaminated before moving, changing filters, or repairing; formaldehyde gas typically will be used for decontamination.

Guideline: NIH Laboratory Safety Monograph; NSF 2002

- C. **Autoclaves:** Autoclave efficacy testing verifies proper decontamination of hazardous wastes before disposal and assures sterility of research reagents and equipment. Autoclaves must be checked for efficacy periodically (monthly, at minimum) by processing a typical load in the presence of an indicator, such as spores of *Geobacillus stearothermophilus*, that integrates time and temperature. Daily cycles may be checked with temperature indicators.

Regulation: Chapter NR 526, Wisconsin Administrative Code (WI Department of Natural Resources Medical Waste Rule)

- D. **Centrifuges:** Mechanical failure of centrifuges can result in aerosol dispersion and high-velocity release of broken parts. Centrifuges must be inspected periodically for rotor wear, cracks, corrosion, and "O" ring deterioration.

Guideline: NIH Laboratory Safety Monograph

- E. **Annual testing of exhaust air filtration systems:**

1. Routine preventive maintenance of filtration systems is necessary to protect building occupants and personnel who service mechanical equipment. All ancillary BSL-3 exhaust air filtration systems must be tested annually for air flow, filter pressure drop, and particle penetration (ANSI 510-1980).
2. Whenever possible "Bag In – Bag Out" HEPA and prefilter change out systems are preferred to help minimize service personnel and environmental exposures during filter changes. Removed filters must be appropriately decontaminated (e.g., autoclaved) before disposal.

Guideline: NIH Laboratory Safety Monograph

- F. **Negative room pressure:** BSL-3 ventilation must be verified to ensure protection of laboratorians and the environment. Negative room pressure will be tested annually and the entire mechanical system air balanced to maintain the inward directional air flow requirement of -0.01 to -0.05 " WC.

Guideline: CDC/NIH BMBL, BSL-3, Section III.D.15

- G. **Laboratory inspections:** NIH and other agencies require documentation of BSL-3 laboratory inspections and certifications. All relevant safety equipment certifications must be in place for successful inspection of a laboratory. Deficiencies must be corrected within a specified time frame to assure that BSL-3 laboratories are providing containment and that personnel and the environment are protected from biohazards.

Guideline: CDC/NIH BMBL, BSL-3, Section III.D.15

- H. **Commissioning** of all BSL-3 laboratories will be conducted prior to occupancy by researchers. The BMBL should be used as a general guidance document, but a lab/research specific biosafety manual is required. The following outlines the steps that will help you define the facility requirements and ensure that its performance is acceptable. These steps are most applicable to new facilities or large remodeling projects, but may also apply to small or single lab remodels.

1. Planning

- a. Project Goals and Expectations (Design Intent)
 1. Risk Assessment - planned usage.
 2. Safety Features - ensure that the users are protected from release of contaminants even in extreme situations.
 3. Flexibility - ease of making changes to the system without excessive costs.
 4. Reliability - system redundancy can prevent dramatic consequences for critical situations.
 5. Economics - cost of requirements may exceed funds available. Compromises can be made only if safety of the users is maintained.
 6. Functional Performance - operational requirements that have to be achieved.
- b. Commissioning Plan - roadmap to meet owner's requirements.

2. Design

- a. Basis of design - codes, guidelines, and standards used; design components and systems.
- b. Construction Documents - integrate commissioning so all parties involved are aware of the quality to be used and expected by the owner.

3. Construction

- a. Mock-Ups - increase quality and reduce installation costs.
- b. Checklists - verify components and systems meet design intent.
- c. System Verification - testing, adjusting and balancing of all systems.

4. Acceptance
 - a. Functional Testing - normal operational testing, as well as all foreseeable abnormal operating conditions (emergency, safety, start-up, shutdown, etc.).
 - b. Controls – testing to verify proper function
 - c. Exhaust Hoods and Systems - performance testing to meet specific acceptance criteria must be documented.
5. Operation
 - a. Documentation - all necessary information to safely operate and maintain the laboratory systems.
 - b. Training - operation and maintenance staff should have ample understanding of the systems during day-to-day operation. Lab staff should also know system's capabilities, limitations and safety procedures.
 - c. Lab Audits - review state of laboratory facility and determine deficiencies.
6. Other (UW Specific, Annually)
 - a. Inspect lab space during construction to ensure envelope is well sealed: walls, floor, ceiling, electrical, plumbing, ductwork.
 - b. Test room pressure degradation (leakage) (see Appendix C). USDA, ARS 242M.1 provides the essentials for this testing. BSL-3 and BSL-3-Ag, 1/2 "wc with 1/4 "wc degradation in 20 minutes.
 - c. Test controls - operation, failure scenarios, local and central audible and visual alarms (coordinate with UW Electric Shop, Metasys Group).
 - d. Test equipment - BSCs, HEPA's, autoclave, sinks, eyewashes, shower, etc.
 - e. Final facility inspection.
 - f. Training - PI, lab, service, support staff.
 - g. Annual maintenance, certification testing and shutdown.
 - h. Test run at BSL-3 practices and procedures with RG1 and RG2 agents.
 - i. Review of research protocol and BSL-3 manual (see Appendix D).

Guideline: ASHRAE Lab Design Guide

Appendix A

Regulations, Guidelines, Standards and Directives

Regulations

Environmental Protection Agency (EPA)

EPA 40 CFR 260 Regulations on Hazardous Waste.

This regulation of the Environmental Protection Agency defines the requirements for disposal and handling of hazardous waste including chemicals, biological agents, animal waste and carcasses.

Nuclear Regulatory Commission (NRC)

NRC 10 CFR 20 Standards for Protection Against Radiation.

This regulation outlines facility standards for research laboratories using radioisotopes.

Occupational Health and Safety Administration (OSHA)

OSHA 29 CFR 1910 Safety Standards.

This Occupational Safety and Health Administration regulation defines minimum laboratory facility requirements for personnel protection. Included are 1910.1450, which incorporates operational and facility requirements for laboratories, and 1910.1030 covering occupational exposure to bloodborne pathogens (HIV and HBV research laboratories and production facilities).

OSHA 20 CFR 1990 Safety Standards for Carcinogens.

This Occupational Safety and Health Administration regulation defines minimum facility requirements to limit occupational exposure to carcinogens.

U.S. Department of Agriculture (USDA)

Wisconsin Department of Commerce (DCOMM)

COMM- 32 Public Employee Safety and Health

COMM - 35 Infectious Agents

COMM - 64 Heating, ventilating and air conditioning

Wisconsin Department of Natural Resources

WI DNR Medical Waste Rule (Wisconsin Administrative Code, Chapter NR 526)

Wisconsin Administrative Code

COMM 64

Guidelines

American Society of Heating, Refrigeration and Air-Conditioning Engineers, Inc.
Laboratory Design Guide - 2001

NIH Design Policy and Guidelines, Spring 2003

National Institutes of Health (NIH), Guidelines for Use of Chemical Carcinogens.
This guideline defines recommendations for dealing with chemical carcinogens for programs with NIH funding including specific facility requirements.

National Cancer Institute (NCI), Safety Standards for Research Involving Oncogenic Viruses. The NCI standard provides minimum facility requirements for NIH/NCI funded grants involving oncogenic viruses.

National Institutes of Health, Guidelines for Research Involving Recombinant DNA Molecules, Federal Register, Amendment Effective April 29, 1999, Federal Register, May 11, 1999 (64 FR 25361). These guidelines provide recommendations on facility design for laboratories doing rDNA research in institutions receiving NIH funding for such research.

NIH Laboratory Safety Monograph. 1979.
This document supplements the guidelines for recombinant DNA research.

Public Health Service, U.S. Department of Health and Human Services, *CDC/NIH. Biosafety in Microbiological and Biomedical Laboratories, 4th edition, May 1999 (CDC/NIH BMBSL-).-Under Revision.* This guideline recommends minimum facility and operational requirements for laboratories working with biological hazards. Also included are animal facility criteria.

Public Health Service, U.S. Department of Health and Human Services, *CDC/NIH. Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 2nd Edition, September 2000*

State of Wisconsin, Division of State Facilities
Laboratory Design Guidelines - 2003

University of Wisconsin-Madison Safety Department,
Laboratory Safety Guide. 2005

University of Wisconsin, Facilities Planning & Management
Fume Hood Policy - 2001

U.S. Department of Agriculture, Agricultural Research Service. Biohazard Containment Design. Chapter 9 242-1m.

Standards

American National Standards Institute (ANSI)

ANSI 510-1980, Nuclear Air Filtration Testing.

ANSI Z358.1, Emergency Eyewash and Shower Equipment.

ANSI Z9.5, Laboratory Ventilation

ASHRAE, American Society of Heating, Refrigeration and Air Conditioning Engineers

ASHRAE 52-76, Test Methods - Air Filters.

ASHRAE Hand Book-Applications-1995, Chapter 43 Sound & Vibration Control,
Page 43.5 Table 2, Design Guidelines for HVAC System Noise in Occupied Spaces-
Research Laboratories with speech communication 40-50 RC.

National Fire Protection Association (NFPA)

NFPA 45, Fire Protection for Laboratories Using Chemicals. 1991.

This code defines facility requirements for laboratories using chemicals for research purposes including university facilities. It includes the requirements for safe design and operation.

NFPA 99, Fire Protection for Health Related Laboratories. 1990.

This code defines facility requirements for laboratories using chemicals in health institutions including clinical laboratories. It includes the requirements for safe design and operation.

NFPA 30, Flammable and Combustible Liquids Code.

This code defines safe storage and handling requirements for flammable and combustible liquids.

NFPA 801, Facilities Handling Radioactive Materials. 1991.

National Sanitation Foundation (NSF)

NSF 49, Biological Safety Cabinets. 2002.

Appendix B

Clean Air and Biological Safety Cabinet Items on UW Contract (05-5770)

Copies available from University of Wisconsin Purchasing Department (262-6335).

Specification UW-MDN-01-05

Class II, Type A1/A2 - Console Biosafety Cabinet Recirculated

Specification UW-MDN-02-05

Class II, Type A2 - Bench Biosafety Cabinet Recirculated

Specification UW-MDN-03-05

Class II, Type B1 - Console Biosafety Cabinet with Hard Connection with 60% Exhaust

Specification UW-MDN-04-05

Class II, Type A2 - Bench Biosafety Cabinet with Canopy Exhaust

Specification UW-MDN-05-05

Class II, Type B3 - Bench Biosafety Cabinet with Hard Connection with 30% Exhaust

Specification UW-MDN-06-05

Class II, Type B2 - Bench Biosafety Cabinet with Hard Connection with 100% Exhaust

Specification UW-MDN-07-05

Class I - Biosafety Cabinet Recirculated, Bedding Dump Station

Specification UW-MDN-08-05

Class II, Type A1 - Animal Transfer Station

Specification UW-MDN-09-05

CAD - Clean Air Device Non-Hazardous, Small Animal Transfer Station

Specification UW-MDN-10-05

CAD - Clean Air Device Non-Hazardous

Specification UW-MDN-11-05

CAD - Clean Air Device Non-Hazardous, Large Clean Animal Transfer Station

Specification UW-MDN-12-05

Class 100 Glovebox Isolator, Negative Air Pressure, Hazardous Pharmaceuticals

Specification UW-MDN-13-05

Class 100 Glovebox Isolator, Positive Air Pressure, Non-Hazardous Pharmaceuticals

Specification UW-MDN-14-05

Class II, Type A2, Large Laboratory Equipment Containment Enclosure

Specification UW-MDN-15-05

Dual Bench, Class II, Type A2 Cabinets with Interconnected Transfer Panels

Appendix C**Testing and Certification of BSL-3 Containment Rooms**

General:

The purpose of testing the containment room or envelope is to determine if the walls, floors, ceilings, penetrations, and other containment barrier features have adequate integrity to prevent leakage of air from the containment space. Testing will be completed by subjecting the containment area to negative air pressure in excess of the anticipated operating conditions, and monitoring the containment air pressure over a test period.

All test procedures must comply with OSHA standards.

Testing and Certification will typically consist of three progressive steps:

- a. Pre-testing for gross leaks by raising/lowering the containment space air pressure to about ½ inch W.C. (125 Pascal), then looking and listening for major leaks.
- b. Soap bubble pre-testing.
- c. Pressure decay testing for final certification.

The general contractor shall be responsible for the coordination and sealing of all penetrations including plumbing, fire protection, HVAC and electrical and the perimeter joints of the containment areas. The general contractor will also be responsible for repairs as needed to comply with the test criteria below.

The testing and balancing contractor shall be responsible for providing the test equipment required to provide and monitor the negative pressures indicated below.

Decontamination piping ports will be available to the Testing and Balancing contractor for vacuum hook-up. All required connections are the responsibility of the Testing and Balancing contractor.

Tests must be repeated until successful results are obtained.

Pre-testing

Prior to testing, supply and exhaust ventilation openings shall be sealed closed, and all doors and other openings through the containment perimeter shall be placed in their normal closed positions. Refer to drawings for details of the sealing of containment suite doors. The door seals detailed shall be provided by the general contractor. Two door seal units will be required for testing of each suite. Once one suite has been tested and verified the door seal units can be relocated to another suite. The general contractor will provide two staff within the confines of the suite for each test to check for and mark the locations of any leaks.

A calibrated digital or inclined manometer shall be installed across the containment perimeter in a manner to minimize interference with wind or ventilation turbulence and to accurately represent the interior and exterior differential air pressure. The manometer shall have a display with capabilities to be easily read to an accuracy of 0.05 inch W.C. (10 Pascal) and capability to accurately read pressures to 3 inches W.C. (750 Pa).

When pre-testing for large/gross leaks, the containment space will be depressurized by installing a variable speed vacuum or other approved means to generate a nominal ½ inch W.C. (125 Pa) differential pressure across the containment perimeter. The building surfaces, joints, penetrations, etc., are then inspected for air leakage and sealed in accordance with the plans and specifications. Note: generating excessive negative or positive pressures can apply significant stress to the facility, and may cause damage that will be repaired at the Contractor's expense.

Following completion of sealing of all leaks identified at ½ inch W.C. (125 Pa), pre-testing may proceed to soap bubble testing. Depending on the location of the containment barrier and construction, soap bubble testing may be completed under positive or negative differential pressure. Testing is completed under negative pressure, when the soap bubbles are readily visible on the inside surface of the containment barrier.

Soap bubble testing

Provide a fan/blower unit with the capacity to create and maintain a 1/2 inch W.C. (500 Pa) differential pressure for the time required to inspect all surfaces and to mark leaks. As the containment zone is sealed, the fan/blower capacity required to maintain adequate differential pressure becomes significantly smaller. A simple shop vacuum unit may be adequate for a large building. Provide a valve or other means of throttling the fan/blower unit to slowly "load" the building with pressure differential, and to keep from creating too large a pressure differential and causing damage to the structure.

Apply a soap or detector solution (e.g., a liquid detergent with a low surface tension, or a commercial test solution such as "Leak-Tek," "Search," or "Snoop") to all joints, corners, sealed penetrations, or other locations which could be point sources of air leakage. Potentially porous construction surfaces such as wood, masonry units, and mortar joints should be carefully checked. Mark all locations of bubble formations and air leaks. Remove the pressure differential and repair the leaks in accordance with the plans and specifications. Following adequate curing time, repeat the soap bubble testing.

Repeat testing and sealing cycles until it appears that the containment zone will pass pressure decay testing. If a ball valve is located in the fan/blower piping from the containment zone, the valve can be closed to seal the containment zone. With the valve closed, monitor the time for the containment pressure to drop from 1/2 inches W.C. (125 Pa) to 1/4 inch W.C. (62.5 Pa). If the time approaches 20 minutes or more, the containment zone should be ready for pressure decay testing.

Pressure Decay Testing and Certification

Final testing shall be witnessed by the commissioning agent and University and State representatives.

Prepare for testing by closing openings at the perimeter of the containment envelope and setting up testing equipment as described for pre-testing. The fan/blower unit shall be capable of creating a 1/2-inch W.C. (125 Pa) pressure differential in the containment zone, and shall have a ball valve in the piping to the containment zone to allow the room/zone to be sealed once the testing pressure differential has been reached.

Testing shall be completed under generally stable conditions of outside wind, temperature, barometric pressure, and humidity. Testing shall be under negative differential pressure with respect to the surrounding environment. Air pressure testing ports/openings for the digital or inclined manometer instruments shall be located where the readings will not be affected by wind, air disturbances, or traffic.

Pressure Decay Testing Procedure:

- a. Operate fan/blower unit to slowly (5 to 10 minutes) bring the differential pressure to 1/2 inches W.C. (125 Pascal).
- b. Close the valve between the fan/blower and the test zone to seal the containment zone at 1/2 inches W.C. negative pressure with respect to the adjacent areas.
- c. Record the differential pressure each minute for 20 minutes.
- d. Slowly open the seal valve to allow the room/containment zone to return to normal pressure.
- e. Decay testing may be repeated after a 20 minute wait period. Visually inspect the containment surfaces between testing and make repairs as necessary. If the acceptance criterion is not met, repeat the soap bubble testing and make repairs before retesting.

Acceptance Criterion:

Two consecutive pressure decay tests demonstrating a minimum of 1/4 inch W.C.(62.5 Pa) negative differential pressure remaining after 20 minutes, from an initial negative pressure differential of 1/2 inches W.C. (125 Pa).

Reports:

At a minimum, reports for each decay test shall include start time, start and end room temperature, date, manometer data (brand, model, serial number, date of last calibration, full scale reading, and smallest scale increment), description of fan/blower unit and control means, tabulation of pressure differential readings for each test minute, a graphical plot of test data (time on the horizontal scale and differential pressure on the vertical axis), a floor plan illustrating the containment envelope and location of the fan/blower unit, and a description of the test, including seals and blockouts. Reports shall be signed and dated by the person completing the test.

Appendix D

BSL-3 Manual - Points to Consider

The purpose of preparing a BSL-3 manual is to provide detailed procedures that staff follow while working in your facility under BSL-3 containment. The manual should be specific to the facility, agents, and procedures used. By providing details about the precautions and procedures actually used, OBS can provide feedback and engage in discussions regarding possible improvements.

The manual should be written so that it serves as a training tool for employees. The instructions must be explicit. Vague statements, such as that “appropriate steps must be followed,” are not acceptable. The manual also serves to meet the requirement stated in BMBL that a manual specific to the agents/procedures/facilities is prepared, and should satisfy the Institutional Biosafety Committee that hazards are being mitigated.

Two documents that should be consulted are

CDC/NIH, Biosafety in Microbiological and Biomedical Laboratories (4th ed) Section III
<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm>

NIH Guidelines for Research Involving Recombinant DNA Molecules, Appendix G-II-C
http://www4.od.nih.gov/oba/rac/guidelines_02/Appendix_G.htm

The following topics should be considered. Some may not apply to your situation. This list is not all inclusive.

Procedures for entry:

Hazard communication signage posted

Who is allowed into the facility? How is a shared facility managed?

Physical security that limits access

Entry requirements (immunization, fitness to wear a respirator, etc.)

PPE that is to be worn (may be different for lab vs. animal rooms and vary by procedures to be conducted) and the procedure for proper donning.

Respirator(s): What type is used? Who does fit testing, medical evaluation to wear a respirator, and training? If reused, how is it cleaned/stored? For what procedures should it be used (lab and animal procedures)?

Training requirements to work in the BSL-3 facility (how often, documented, ...)

Routine procedures/precautions

What containment methods are used for various procedures?

Is plasticware substituted for glass?

Handling of sharps (generally discouraged in BSL-3 facilities)

Precautions used when aerosols might be generated outside BSC, e.g., centrifugation and flow cytometry.

Precautions/containment used if mixed hazards present (e.g., biological and chemical)

Are staff allowed to work alone at night and weekends (generally discouraged)?

Is a hand washing/hands-free sink available?

Are the ventilation monitoring systems checked and readings recorded?

Precautions used for vacuum lines (e.g., filters or disinfectant traps)

Autoclave efficacy testing (how often and method)
Precautions used during necropsy
Housekeeping procedures (how often? who is responsible?)

Routine maintenance and repair procedures:

Surface cleaning (what disinfectant is used, concentration, how often is it done)
Who washes the floor and how often; if tile floor, how is impervious surface maintained and by whom?
Who is responsible for flushing the eyewash?
Procedures for allowing custodians and repair workers into facility - Are they accompanied at all times? Are they informed of hazards and what they should stay away from? Will their equipment need to be surface decontaminated prior to exit?
Procedures to be used if equipment needs to be sent out for repairs.

Equipment/Facility certification, maintenance, and testing

Annual shutdown of the facility to certify, repair and test facility and equipment
Procedures for decontamination prior to facility work beginning
Annual certification of BSCs and facility HEPA filters (required)
Annual inspection, maintenance/repair and testing of facility: interior surfaces (floors, walls, ceiling, bench tops), plumbing, electrical, specialized HVAC system and controls) to verify containment.

Procedures for exit

Details for removing PPE, disinfecting and/or discarding it
Waste removal/disposal
Laundering of lab coats
Precautions used if/when materials are removed from the BSL-3 facility, e.g. papers and viable or fixed samples.

Animal handling – responsibilities (animal care staff, researchers, others)

Precautions for administering pathogens to animals
Type of cages/cage systems used
Bedding changes
Cage cleaning – autoclaved prior to washing?
Animal and animal waste disposal – autoclaved and/or incinerated?

Emergency response

What constitutes an emergency and what are the procedures for dealing with it?
What if your BSC, incubator, centrifuge malfunctions?
What if the room pressure alarm goes off? ...the lights go out? ...there is a needle stick?
What if the infected animal gets loose? Are there other worst case scenarios?
Who should be contacted?
Should individuals carry information regarding the nature of the pathogen that they handle?
What does it mean when an alarm sounds? How should staff respond to an alarm?
Provide a copy of the spill procedure(s) to be posted in the facility.

Where are the nearest eyewash and emergency shower located?

Provide a hierarchical contact list that your personnel could use for notification when you are unavailable in case there is an incident such as suspicion of exposure.