

BL-3 Level Research

All personnel involved in Biosafety level 3 research must be appropriately trained by the Institutional Biosafety Officer and the Laboratory Director, according to the Guidelines before beginning the proposed research. Training must be documented. Individuals conducting research requiring BL3 containment and practice must attend a tutorial session provided by the Biological Safety Officer (BSO) and pass an Institutional certifying examination administered by the IBC/BSO. Investigators planning animal experiments requiring BL3 containment must also satisfy the requirements of Lab Animal Science and the Institutional Animal Care and Use Committee (IACUC).

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I. Introduction

These procedures describe the facilities and operating methods necessary for the safe handling and containment of infectious agents and/or recombinant DNA molecules requiring Biosafety Level-3 (BL-3). The BL-3 laboratories are located in room 324 Jefferson Alumni Hall (JAH), room 722 Bluemle Life Sciences Building (BLSB), and the BL-3 animal facilities on the 3rd floor of JAH and the 11th floor of BLSB.

The Standard Operating Procedures (SOP) were designed to meet the requirements for working with BL-3 level infectious agents as published in:

1. The CDC/NIH handbook, *Biosafety in Microbiological and Biomedical Laboratories*, 4th edition (1999);
2. *Working Safety with HIV in the Research Laboratory*, Morbidity and Mortality Weekly Reports (Supplement 4), 37: 1-19, (1988);

3. NIH Guidelines for Research Involving Recombinant DNA Molecules, 51FR, 19658, (1986);
4. OSHA Bloodborne Pathogens Standard, 29CFR 1910.1030.

II. Access to Facilities

In order to have access to the facilities, personnel must have specific training in aseptic technique as applied to biological work. This includes tissue culture. They must know the facilities and practices required for working with infectious agents and/or recombinant molecules either in vitro or in vivo requiring BL-3 containment conditions.

Personnel will take a written examination to document knowledge of the facilities and procedures. A mini-course designed to prepare personnel will be given jointly by the Department of Environmental Health and Safety (EHS), the Institutional Biosafety Committee (IBC) and the Office of Animal Resources (OAR). The course will cover procedures to meet the requirements for working safely in a BL-3 laboratory. Only experiments and procedures approved in advance by the IBC and/or the Institutional Animal Care and Use Committee (IACUC) are permitted to be performed in the facilities.

Entrance to the facility by non-research personnel requires approval by the Manager of the facility. The Manager shall accompany such individuals during their stay at the facility.

An emergency call list will be posted at the entrance to the containment area identifying key personnel, laboratory and home phone numbers.

A log of those entering and leaving the facility will be kept and shall include the name, time present in the facility and a notation of any problems that might have occurred during that time. Key card access records on computer may be used in place of a written log.

III. Facility Manager

Each BL-3 laboratory or animal facility shall have a Manager who is a senior faculty member appointed by the Chairman of the department or Director of the Division where the facility is located. The Manager shall be fully knowledgeable about the regulations cited in the Introduction and about infectious agents requiring BL-3 facilities and practices. Work experience and formal education will meet this requirement.

IV. Responsibilities of the Manager

1. Overall supervision of the facility
2. Oversight of maintenance of equipment and safety cabinet certification
3. Supervision of emergency situations
4. Determination of a researcher's training and qualifications
5. Documentation and correction of any minor lapse on the part of any investigator or staff in BL-3 practice

6. Report to IBC any flagrant violation of BL-3 practice on the part of any investigator or staff member
7. Maintenance of a list of agents under study at the facility, their infectivity and pathogenicity.
8. Maintenance of a list of appropriate disinfectants to use. This information shall be communicated to all investigators in the facility and entered into the emergency response protocol.
9. Provision of a semi-annual report to the IBC on the activity in the facility and any problems during that period.

V. Training of Investigators

All new investigators must undergo a period of training provided by the EHS, IBC, and OAR. This training includes aseptic technique and the practice of tissue culture, an understanding of the BL-3 containment conditions and practice and the proper use of a biological safety cabinets, autoclave and other equipment in a BL-3 facility. Proper waste handling procedures and emergency procedure will also be included in the training. Classification and properties of the infectious agents being studied by the multi-users of the facility will be covered, as well as the particular problems associated with the use of infectious agents in animal studies. Users of the facility will have to pass a written exam to demonstrate that they have acquired the appropriate knowledge base.

VI. A BL-3 Facility

Biosafety Level-3 is applicable to clinical, diagnostic, teaching, research or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent and experienced scientists. All procedures involving the manipulation of infectious materials are conducted within a biological safety cabinet by personnel wearing protective clothing and equipment.

The laboratory has special engineering and design features. Personnel employ universal precautions. The special features of a BL-3 lab and the safety practices to be followed are described in the next sections.

A. Special Features of a BL-3 Facility

1. A Class II biological safety cabinet must be used:
 - a. It provides protection for both worker and experiment.
 - b. Must be properly used to be effective.
 - c. Standard microbiological techniques must be used.
 - d. Exhaust air, after passing through a high efficiency particulate air (HEPA) filter, exits directly to the outside or through the building exhaust system.

- e. The air passing through the air duct is monitored by an airflow monitor, which will activate an alarm if there is an exhaust fan failure.
 - f. The cabinet is inspected by an outside firm at least once a year but preferably twice a year.
 - g. Cabinet should run continuously.
 - h. If the fan in the cabinet is turned off, it should be turned on at least 10 minutes before working with an infectious agent and allowed to remain running 10 minutes after working with an infectious agent.
2. The walls, ceiling, floors and base boards are painted with a special epoxy based paint which is resistant to water and can be easily decontaminated. The bench tops must be resistant to water, acids, alkalis, and organic solvents. All bench tops and cabinets must be sealed where they abut the walls. If there are any windows, they must be sealed also.
 3. The fixtures on the laboratory sinks must be foot or elbow operated.
 4. Two sets of doors separate the BL-3 laboratory from the corridor or main laboratory. The doors cannot be opened at the same time; they are self-closing, and the outer door is self-locking.
 5. The entire BL-3 laboratory is under negative pressure relative to the adjacent room(s) and/or corridor(s). Air must always flow from the least contaminated area into the BL-3 laboratory. Room air is exhausted directly to the outside.
 6. All centrifuges must contain gasket-sealed lids. Screw-capped safety centrifuge tubes must be used for infectious materials.

B. BL-3 Practice in the Facilities

Anyone who is unsure about the safety procedures to follow for any circumstances must consult with the Manager before proceeding.

1. Doors to the BL-3 laboratory must be kept closed and locked at all times:
 - a. To provide security.
 - b. To maintain proper negative pressure in BL-3 containment.
2. All non-research personnel (such as maintenance or service personnel) must be accompanied by an authorized person during their stay in the unit. In order to enter the facility, the following may be needed:
 - a. Documentation of proper immunization (if needed).
 - b. Instructions on how to exit and enter the unit properly.
 - c. Special instructions for cleaning and disinfecting tools after use.

Before entry into the BL-3 laboratory, all instruments, equipment and biological safety cabinets must be disinfected or sterilized before repairs and inspections can take place.

3. Eating (including gum chewing), drinking, applying cosmetics, mouth pipetting, possession of any type of food and books not related to the work being performed, are forbidden in any BL-3 facility.

C. Minimum Protective Requirements

Before entering the BL-3 facility, remove lab coat and all pencils, pens and personal valuables.

1. Anyone entering the facility to work with biological or infectious material or to service equipment must wear the following personal protective equipment:
 - a. disposable gown (tied in back) or jumpsuit
 - b. face mask
 - c. head covering
 - d. foot coverings
 - e. goggles or protective eyewear
 - f. double gloves

Don the first pair of gloves before the gown. After gowning, don a second pair of gloves which must overlay the gown so the wrist area is completely covered. Outer gloves must be taken off when answering the phone and changed when contaminated or ripped.

Open wounds, chapped or bleeding hands require that you stop both BL-2 and BL-3 experiments involving bloodborne pathogens until the skin heals.

2. Personnel entering the BL-3 facility to do repairs or cleaning are required to observe the minimum appropriate protective requirements. Depending on the job, this may or may not require all of the items listed in number 1. Personnel having only administrative duties within the BL-3 Laboratory (at a time when samples are not being processed), may wear the minimum of shoe covers, disposable gown and gloves.
3. All work with infectious material must be performed in a biological safety cabinet, except centrifugation (see section on centrifugation.)
4. Clean up when you have completed your experiments!

The disinfectant that you use will depend on the biohazardous agent. The following disinfectants are approved by the Centers for Disease Control:

- a. 10% Clorox or Betadine
- b. Amphyl (phenolic)

- c. Glutaraldehyde
- d. 70% alcohol

Seventy percent alcohol is not recommended by the CDC as a stand-alone surface disinfectant. The contact time is not long enough because of rapid evaporation. It is acceptable as a wipe-down agent to remove residue from a prior disinfection. Amphyl or Wescodyne can be used in place of alcohol as a surface disinfectant.

Wipe bench and biosafety cabinet hood surfaces with an appropriate disinfectant. Autoclave all used material and all trash (including empty boxes). Rinse all glassware with Clorox and then wash thoroughly. Soak hemacytometers for 5 minutes in a 10% solution of Clorox and rinse well. Each person is responsible for their own trash

- 5. Biosafety cabinet cleanup is particularly important!
 - a. All pipettes and pipette tips are placed into the Clorox-filled pan either under or in the safety cabinet. (preferably in the cabinet)
 - 1) Upon completion of the work within the cabinet, the pan should be allowed to sit for 20 minutes after the last pipette/pipette tip has been placed in it.
 - 2). The Clorox can then be poured down the drain; the pipettes and/or tips placed in an autoclave bag, and the pan itself can be autoclaved.
 - b. All liquid waste including culture medium, supernatants, and other infectious liquid waste, is vacuumed off or transferred to a flask containing Clorox so that the final concentration of the Clorox in the flask is 10%.
 - 1). Allow to sit for 20 minutes after the completion of the work
 - 2). Pour the contents down the sink.
 - 3). Autoclave the vacuum flask
 - c. All solid waste must be decontaminated with Clorox and then put into an autoclave bag. (Make sure there is no residual Clorox so as to avoid creating Chlorine gas upon autoclaving)
 - d. Tissue culture flasks and all other solid waste go into an autoclave bag located next to the biosafety cabinet.
 - e. All infectious material or materials that have come in contact with infectious material are placed in autoclave bags before leaving the biological safety cabinet.
 - f. Spray bottles containing 10% Clorox or Wescodyne are to be kept in the biological safety cabinet.
 - g. When clean up is complete, remove your outer pair of gloves while still under the biosafety cabinet, then autoclave the pan (after removing the Clorox) and bag.

6. Infectious materials (such as virus stocks) which are to be stored, are to be placed in durable leak-proof containers (screw-capped tubes or vials). The tops must be closed and the outer surface of the tubes should be sprayed with appropriate disinfectant before removing them from the biosafety cabinet.
7. All specimens of blood, tissues, cells and body fluids of a potentially infectious nature (HIV and HBV etc.) from human or other species that are for storage elsewhere, are to be transported in a secure container with a lid. These specimens must be unwrapped and worked on within the biosafety cabinet.
8. Any hazardous chemical waste will be handled by Environmental Health and Safety.
9. Autoclaving:
 - a. Sterilization of infectious liquid (**NOT** containing Clorox) and solid waste is carried out by autoclaving at 121 degrees for 60 minutes.
 - b. Autoclave bag must be put into a pan for autoclaving.
 - c. Water must be added to the autoclave pan.
 - d. A sterilization indicator strip must be included in each autoclave run.
 - e. Perform a spore challenge in the autoclave on a weekly basis.
 - f. Autoclave cycles must be monitored.
 - g. Waste (after autoclaving) must be put into a red bag, and then boxed. This should be done only after the day's activities are completed and biohazardous materials have been put away with the necessary decontamination procedures completed.
10. Biological Safety Cabinets (BSC):
 - a. All procedures that are known to create or have the potential to create an aerosol must be carried out within the BSC.
 - b. **All in-house vacuum lines must have a trap containing Clorox or other suitable disinfectant and an in-line bacterial filter.**
 - c. A mortar and pestle may be used to emulsify tissue. Glass tissue grinders or blenders must not be used.
 - d. Use plastic-backed absorbent pads to cover the cabinetwork surface to facilitate clean up of spills.
 - e. The grillwork within the BSC must not be blocked. This will affect airflow and possibly compromise safety.
 - f. Disinfect the BSC after all procedures.

11. Absolutely NO needles or glass pasteur pipettes are allowed in the *in vitro* BL-3 facilities!

- a. Disposable polypropylene transfer pipettes are permitted.
- b. Needles may be required for animal work and may be used after adequate consideration is given in the protocol to avoidance of needle sticks.
- c. Use of glass vessels should be kept to a minimum.
- d. Unbreakable disposable plastic is recommended.
- e. Pipetting by mouth is strictly forbidden!

12. Radio labeled infectious material:

- a. This material must be disinfected first and then considered as radioactive waste.
- b. Solid and liquid radioactive waste must be stored in an appropriate container in a holding area according to the requirements of Radiation Safety.

13. Centrifugation of infectious or potentially infectious material:

- a. Table top centrifuge
 - 1). All blood, body fluids or cells containing infectious or potentially infectious agents are placed in screw-capped, leak-proof tubes.
 - 2). The outer surface of the tube is sprayed with disinfectant before transfer to the centrifuge.
 - 3). After centrifugation, the inner surface of the tube holders must be thoroughly decontaminated with an appropriate disinfectant.
- b. Ultracentrifuge
 - 1). Samples for ultracentrifugation must be prepared in the biosafety cabinet.
 - 2). A gown, gloves, facemask and goggles or a face shield must be worn when working with these samples.
 - 3). Tubes must be tightly capped.
 - 4). The outer surfaces of the tubes must be decontaminated after filling and before placing in the rotor.
 - 5). Make sure the O rings for the rotor are in good condition and properly greased to seal the rotor.
 - 6). The rotor should be sprayed with disinfectant before it is removed from the biosafety cabinet. The investigator must either spray their gloves or change them before taking the rotor out of the biosafety cabinet.

- 7). After centrifugation, in order to prevent possible aerosol, the rotor must be removed and put in the biosafety cabinet. The rotor can then be opened and unloaded.
 - 8). Decontaminate the tubes, rotor chamber and outer surface with disinfectant.
14. Virus concentration and/or purification using procedures such as gradients or filtration may generate a great risk of virus exposure. A gown, gloves, facemask, goggles or a face shield must be worn.
15. When the work is finished in the BL-3 laboratory:
- a. **Remove outer gloves first!**
 - b. Remove all protective clothing and place into an autoclave bag located by the door of the Containment Suite.
 - c. Remove shoe coverings.
 - d. **Remove inner gloves last!**
 - e. Leave the Containment Suite and wash hands in the anteroom before leaving the facility.
16. All waste must be autoclaved prior to disposal. Autoclaved bags are then put into a box lined with two red biohazard bags near the autoclave. When the box is full, follow the instructions provided by the Department of Environmental Health and Safety.

VII. Emergencies

Emergencies which are very obvious would include large spills, ventilation failure, alarms sounding, power outages, centrifuge crashes and fires. Other emergencies that may not be as obvious include steam leaks in the autoclave, freezer failure, gas leaks and others. However, within the BL-3 facility, an emergency shall be defined as any occurrence that is not normal.

In the event of an emergency or an abnormal event:

1. All work shall cease.
2. Personnel within the facility must leave.
3. Dial 811 on the nearest phone to notify Security.
4. Security will notify the Emergency Response Team and any appropriate personnel.
5. Environmental Health and Safety, Facilities Services, Animal Resources and the Institutional Biosafety Committee will respond to the facility.

A. Emergency Procedures for a Fire

Fires within a laboratory where biohazardous agents are being stored will require an immediate response. This will minimize personal exposure and limit the potential spread of biological contamination. Fires may create toxic smoke as well as aerosols that may contain infectious material. Fire and/or the sounding of the alarm must be treated with equal importance. Personnel are required to leave the containment facility and follow all fire notification/evacuation procedures.

B. Emergency Procedures For An Accident

The response for a major spill outside the biosafety cabinet, splashes or other incidents, **is to stay calm and think!** The severity of the accident will be judged by the type of accident that occurs.

1. An accident which creates an aerosol

and potentially releases a pathogenic agent is the **most severe**. This especially occurs during a centrifuge accident. Occupants of the room should do the following:

- a. Hold your breath!
- b. Immediately evacuate all personnel within the containment.
- c. Carefully remove potentially contaminated clothing and place it in the autoclave bag by the door as quickly as possible.
- d. Wash all exposed body areas with a suitable approved disinfectant. (Example: Betadine surgical scrub or Hibiclens for those allergic to iodine). There is a bottle reserved for this purpose in the anteroom.
- e. Leave the facility.
- f. Notify Laboratory Manager.
- g. Call "811". This will notify the Emergency Response Team.
- h. Do not enter the BL-3 facility for **at least** 30 minutes to allow the aerosolized particles to settle.
- i. Prepare a large volume of 10% Clorox solution.
- j. Personnel involved in decontamination must wear non-absorbent coveralls, double gloves, shoe coverings, head covering, and a face mask. These items are stored in the facility.
- k. Spray all surfaces and items in the laboratory in a careful, methodical manner using the 10% Clorox solution. Include ceilings, walls, bench surfaces, cabinets and floors. Then re-spray everything with 70% isopropyl alcohol.

- I. Remove protective gear and place in autoclave for sterilization. Start autoclave and leave the room.
- m. Decontamination using formaldehyde gas or liquid will be determined by the emergency response team.
- n. Repeat the washing of exposed body areas with a suitable decontaminant.

2. **Accidents involving personnel**

If any personnel are involved in an accident, follow-up medical evaluation, immunosurveillance and medical treatment, may be needed. All accidents resulting in personal injury or exposure will require completion of a University Accident Report form.

C. Emergency Procedures For Spills

1. Response to body exposure to virus and/or human blood, cells or tissues:

- a. Wash area(s) affected by the accident for 5 minutes with a Betadine solution. Do not use Clorox!
- b. Wash area(s) with copious amounts of water or saline solution.

If a large spill and aerosol were also involved in this exposure, activate the emergency procedure described above.

2. Response to a minor spill outside Biosafety Cabinet:

Minor spills and splashes (i.e. cultures etc.) that occur outside of the biosafety cabinet are handled in the following manner: Occupants of the room should use their educated judgment as to whether the spill is minor and whether an aerosol was produced. If the spill is small and no aerosol was likely produced proceed as follows:

- a. Perform Physical Cleanup.
 - 1). Wear protective clothing and double gloves.
 - 2). Contain the spill by covering with paper towels.
- b. Disinfect the spill.
 - 1). Use fresh solutions (Clorox, Alcide and Wescodyne lose strength with time. Make most disinfectant solutions daily. Amphyl is more stable.)
 - 2). Flood spills with appropriate disinfectant, using care not to cause spatter.
 - 3). Add disinfectant slowly to outer margin of spill and allow it to flow in.

- 4). Allow disinfectant to act for 20 to 30 minutes before cleaning up with more paper towels.
 - 5). Discard the contaminated paper towels in an autoclave bag along with the first pair of gloves. (put on new outer gloves)
 - 6). Follow this initial clean up and disinfection with a normal clean-up and disinfection procedure.
 - 7). Place all material used in the clean up in an autoclave bag along with any disposable clothing. Discard gloves. Wash hands and face with disinfectant solution.
- c. Notify the facility manager.

3. Response for small spills inside the biosafety cabinet:

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet. Be careful with paper towels which can be sucked into the blower fan or HEPA filters.

- a. Spray or wipe walls, work surfaces and equipment with an appropriate disinfectant.
- b. If spill dripped down on to the drain pan below, (if using a Class II Cabinet) flood the top work surface tray and drain pan with an appropriate disinfectant and allow to stand 20 minutes.
- c. Remove excess disinfectant from the tray by wiping with a sponge or cloth soaked in a disinfectant. For Class II cabinets, drain the tray into the cabinet drain pan, lift out tray and removable exhaust grillwork, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a disinfectant. Then replace the grillwork and drain disinfectant from the drain pan into the appropriate container and either autoclave or dispose according to standard procedures. Gloves, cloth or sponge should be autoclaved and discarded into an autoclave bag.

D. Procedure for Mechanical Emergencies in The Facility

A list of all mechanical controls and their locations will be posted at the entrance to the facility. In addition, the Manager shall make certain that all investigators are familiar with the gas, water, and steam shutoffs, the use of the Manual damper control, where present, and the operation of the negative pressure ventilation system.

Specific procedures for the notification of maintenance will be followed. The goal is to have the investigator take care of the immediate problem and decontaminate the facility so that maintenance can correct the problem on a priority basis the next day.

E. Shutdown Procedure

Brief guidelines for shutting down operations during an emergency or evacuation should be posted by the Manager. In so far as possible, biohazardous agents should be secured in a cabinet to minimize danger of breakage, spillage, etc.

F. Start-Up Procedure

The Manager shall establish procedures to ensure that personnel do not return to the facility until the danger from the emergency has ended. Start-up procedures that may be required for some operations should be posted.

G. Persons Security Will Notify to Respond

Emergency Contact Personnel

Emergency Response: 811

Security will notify one or several of the following persons in case of an accident or other emergency in the BL-3 Facility:

Environmental Health and Safety:

Sue Gotta, Biological Safety Officer, ext. 3-7422; cell phone 215-828-2102; home phone 215-493-2235.

Jolene Shaw, Director, Environmental Health and Safety, ext. 3-5852; beeper 215-314-1948; home phone 856-753-4528.

Managers of the BL-3 Facilities and Other Support Personnel

324 JAH

Edward Acheampong, Ph.D., ext: #3-9061 or #3-8575

722 BLSB:

Michael Root, M.D., Ph.D., ext: 3-4564;

BL-3 Animal Facilities, 11th floor BLSB, 3rd floor JAH

Judy Daviau, D.V.M., beeper 215-314-3905.

Facilities Services: 5-6846

VIII. BL-3 Class Infectious Agents and Animal Experiments

There are additional requirements when administering an infectious agent to animals. The investigator doing animal experiments involving RG-3 infectious agents must consult the

Standard Operating Procedures developed by the Office of Animal Resources (OAR) and the Institutional Animal Care and Use Committee (IACUC) for work with that particular agent.

IX. Animal Research Biosafety Facilities

The BL3 animal containment facilities on the 11th floor of Bluemle Life Science Building and the 3rd floor Jefferson Alumni Hall are designed and operated in accordance with the CDC-NIH Guidelines: *Biosafety in Microbiological and Biomedical Laboratories*. These facilities are used to house animals and conduct experiments at the Biosafety Level 3 and for studies involving hazardous agents or requiring special containment or isolation.

A. Operating Principles and Rules

1. Access to Facility
 - a. All personnel must comply with written standard operating procedures and policies for Biosafety 2/3 animal facilities and receive appropriate training.
 - b. Permission to access these facilities is dependent on demonstrating adequate training and knowledge of the facility procedures.
 - c. Must have approval by the Manager of the facility as outlined in this document.
2. Entering the Facility
 - a. Entrance to the facilities is permitted only through the double door entryway and/or locker rooms as described in the standard operating procedures.
 - b. Specific personal protective clothing must be worn upon entering the facilities.
 - c. Supply carts with the necessary items including gowns, foot covers, gloves, and facemasks are provided at the entrances.
 - d. Instructions for appropriate garbing and access procedures are posted.
3. Animal Housing and Containment
 - a. Animals will be housed in microisolator cages or other primary containment units under barrier conditions.
 - b. In most cases, microisolator cages are maintained in HEPA filtered environmentally controlled cubicles.
 - c. Animals are handled only under appropriate Biosafety hoods.
 - d. Refer to standard practices in the animal containment rooms.

4. Animal Care and Husbandry
 - a. Management and Operation of the animal facilities is the responsibility of Laboratory Animal Services (LAS).
 - b. Specific written standard operating procedures govern the animal care and husbandry, sanitation, and movement of supplies, racks, cages, and animals.
5. Working in the Facility: Technicians and Investigators
 - a. The entire facility is considered a biohazardous area. Each animal room and/or cubicle is an isolation unit and may require additional safety practices or procedures, which will be posted. This will be dependent on the pathogens and experiments in process.
 - b. All cage changing and experimental manipulations will be conducted in a Class II, type A/B3 or Class II, type B2 biosafety cabinet depending on the level of containment required.
 - c. Attention must be given to safe working procedures involving wearing of protective garments, minimizing aerosols or other exposures to pathogens, decontamination and containment practices at all levels.
6. Decontamination and Waste Disposal
 - a. All equipment, cages, waste and supplies must be decontaminated by sterilizing in the autoclave before leaving the facility.
 - b. A decontamination room is available for chemical decontamination if required.
 - c. Cidox or Clorox or other approved disinfectants are used on work surfaces in some areas.
 - d. Disposable waste must be placed in autoclave bags and autoclaved/incinerated in accordance with pertinent federal, state and local laws and regulations.
7. Exiting the Facility
 - a. Protective garments, shoe covers, gloves, head covering and facemask must be removed at the exit area in the corridor of the Biosafety Suite.
 - b. A specific line on the floor designates the demarcation between biohazardous facility and exit corridor where a large infectious waste container is located for discarding the garments.

- c. You must exit only in one direction through the respective locker rooms.
- d. Wash your hands before leaving the facility.

Showers are available in the Jefferson Alumni Hall facility.

X. Recommended Disinfectants in BL-2 and BL-3 Labs

A. Alcohols

Classified as intermediate strength disinfectants for immersion only
Alcohols are specifically NOT recommended for solid surface disinfection.

- 1. Ethanol will kill the spectrum of viruses if immersed long enough.
- 2. Isopropyl alcohol will miss some non-lipid viruses such as Adenovirus.
- 3. Alcohols have a problem if there is protein material present. They form a coagulum that prevents penetration to the deeper layers.

B. Recommended intermediate strength disinfectants for BL-2 and BL-3

- 1. Personnel should know the specific agent to be eliminated and study inactivation kinetics for each disinfectant to pick best one.
- 2. When specific data is not known or trusted, the three generic choices are:
 - a. Chlorine containing compounds
 - 1). Clorox 1:10
 - 2). Alcide disinfectant (Chlorine dioxide)
 - b. Phenolics
 - 1). Amphyl (or Beaucoup) is a good choice (order from Hospital Pharmacy)
 - c. Iodine containing compounds
 - 2). Wescodyne disinfectant solution is a good choice
 - d. Another thing to look for on all disinfectant cleaning or spray commercial agents is that it be "Registered with the EPA with a specific label claim for tuberculocidal activity".
 - e. Aqueous alcohol solutions can be a good agent to clean a surface to remove phenolics if a sticky residue gets to be too much of a problem. Aqueous alcohol solutions are also good to remove Clorox or Alcide after 5 minutes of contact time to prevent oxidation of metals and plastics.

3. Handwashing agents
 - a. Iodophor surgical scrubs are best.

XI. The Bottom Line

Remember, each individual is responsible not only for his/her own safety when working in a BL-3 facility, but also for the safety of all persons who enter the facility and all those who come in contact with materials he/she has generated.