

BSB 653A Biosafety Level II (BSLII) Facility Standard Operating Procedures

W. Scott Argraves, Ph.D., Professor

These operating procedures are not intended as a substitute for the MUSC OSHA Guidelines.

Biosafety Level II is similar to Biosafety Level I but is also suitable for work involving agents of moderate potential hazard to personnel and the environment.

Initials:

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1. Eating, drinking, smoking, and the application of cosmetics including lip balm (Chapstik, Blistex, Vaseline, etc.) are not permitted in the facility. Avoid touching fingers to eyes (including contact lenses) or mouth to decrease risk of accidental mucosal exposure. Personal items (i.e., backpacks, briefcases, purses) are not allowed in the facility. Keep the door of the facility closed when work is being conducted.

2. Disposable lab coats are required when working with BSLII agents. Lab coats must be removed before leaving the facility and are to remain in the facility until discarded in the red biohazard bin (orange bag) located within the facility. Lab coats must not be stored in direct contact with street clothes or other personal items used in the facility (i.e., notebooks).

3. Gloves, safety glasses, and masks (PPE) are not required for working under BSLII conditions, however, gloves are recommended if the skin on the hands is broken, if there is a rash present, when handling experimental animals, and when skin contact with the agent is unavoidable. Safety glasses are required when conducting procedures that might generate splashes of microorganisms or other hazardous materials (sonicators, homogenizers etc.). Persons who wear contact lenses are required to wear safety glasses. PPE must be removed before leaving the facility. Reusable PPE (safety glasses) must not be stored in direct contact with street clothes or other personal items (i.e., notebooks). Disposable PPE (gloves, masks) must be disposed of in the red biohazard bin (orange bag) located within the facility. Wash hands with instant hand sanitizer (dispenser located by the water bath) after handling contaminated or potentially contaminated materials, after removing gloves, and before leaving the facility. Wash hands thoroughly with soap and water immediately after leaving the facility. Maintain an alternative set of clothing at the work place in the event street clothes become soiled or contaminated.

4. Decontaminate the counter at the beginning of work and after work has concluded. Apply a liberal amount of 70% ethanol to the counter, spread across the surface, and allow to air dry for a minimum of 15 minutes. In addition, the counter must be decontaminated in the same fashion following any spill of viable material.

5. Use pipetting devices; mouth pipetting is prohibited.

6. Avoid creating aerosols or allow them to settle before opening tubes or containers.

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Vigorous shaking, blending, mixing, or vortexing creates aerosols.

7. Discard all sharps (glass pipettes, tips, needles, blades etc) in the sharps disposal bin located in the P2 containment hood.

8. Discard plastic pipettes in the autoclavable pipette receptacle located inside the P2 containment hood. When this receptacle is full, close the top and place it in the red biohazard bin (orange bag) located within the facility.

9. Discard all other contaminated and potentially contaminated materials in the red biohazard bin (orange bag) located within the facility.

10. **When a red biohazard bin (orange bag) or sharps container is full:** Tape the orange bag or sharps container closed and place in a leak-proof autoclavable container. Label with autoclave indicator tape and autoclave for 30 minutes. Place the autoclaved orange bag in a red biohazard bag before placing it in **the large red biohazard transport bin located in the lab (the autoclaved sharps container can go directly into the bin) for transport to the Biohazard Waste Storage Room located downstairs in the Basic Science Building. Use the east-most service elevator of BSB to get to this room. This room is accessible by card key (activated employee ID).** If the orange autoclave bag "disappears" before autoclaving, page Lisa Steed 2-0590, pager 14463, since the missing bag must be located and disposed of properly.

11. Conditioned culture media must be decontaminated prior to disposal by exposure to household bleach (1 part bleach to 9 parts contaminated solution) for a minimum of 15 minutes:

The vacuum disposal system is made up of 2 waste receptacles and a secondary safety reservoir. * The two waste receptacles are used interchangeably (the empty one is the spare). When one is full, disconnect it from the vacuum system and connect the empty one in its place: to change the full waste receptacle, carefully disconnect it from the vacuum system and add 385mls bleach to the waste. Use the cap from the second (spare) waste receptacle to recap the full receptacle. Now, add 385ml bleach to the spare receptacle and connect it to the vacuum system. Conditioned culture media must be decontaminated prior to disposal by exposure to household bleach (1 part bleach to 9 parts contaminated solution) for

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a minimum of 15 minutes. Pour appropriately decontaminated liquid waste in the toilet. Flush with copious amounts of water.

*The waste reservoir should NEVER be filled above the fill line and must NEVER overflow into the secondary safety reservoir. If the safety reservoir and/or the vacuum lines running in to or out of it are contaminated with conditioned culture media, they will have to be immediately decontaminated by exposure to household bleach (1 part bleach to 9 parts contaminated solution) for a minimum of 15 minutes prior to using the vacuum system.

12. Fire extinguishers are located just outside the door of the main laboratory entrance and just inside the door of the rear laboratory entrance.

13. An eye wash station, hand-washing sink and first aid kit are located on the left side of the main laboratory (BSB 653) when *leaving* the BSLII facility.

14. In case of a spill or accident, identify the area of contamination. Take precautions to prevent spreading the contamination: are your lab coat and/or gloves contaminated, how about clothing not covered by PPE (shoes and the soles of your shoes in particular)? Do you need to disinfect the bottom of your shoes using 70% ethanol?

Procedure for clean up:

- Never touch broken glassware with your fingers. Place absorbent material AROUND the perimeter of the spill/broken glassware to absorb decontaminated solution.
- Pour 1 part household bleach for every 9 parts contaminated solution over the contaminated area. Allow it to sit for a minimum of 15 minutes.
- Use the brush and dustpan located on the shelf under the centrifuge to collect the now decontaminated broken glass and absorbent material.
- Dispose of the broken glass and absorbent material in regular laboratory glass trash.
- Wait! Do you need to change into the alternate set of clothing kept in the lab for this purpose? If so, disinfect contaminated clothing by exposure to 70% ethanol for a minimum of 15 minutes. Once decontaminated, clothing can be washed with regular laundry.

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Immediately notify Dr Argraves of any exposure or contamination (843-697-5328). Dr. Argraves will confirm the status of the exposure. An exposure incident report will be opened and the individual will be taken to either Employee Health (57 Bee Street, 792-2991) or Student Health, (30A Bee Street, 792-3664). Dr. Argraves will also inform the MUSC University Biosafety Office (Dr. Dan Eisenman, 792-3055), and ultimately the NIH Office of Biotechnology Activities (301-496-9838).

Proficiency testing will be done prior to any personnel working with any BSL-2 agent. Training will occur as needed, as is the case with new employees, or when procedural or policy changes occur. Additional training sessions will take place at least annually to ensure lab personnel is adequately trained regarding duties, potential hazards, safety procedures, and exposure evaluation procedures. Attendance at these training sessions is mandatory. Employee signatures will be required to document attendance. It is the employee's responsibility to notify Dr. Argraves at any time of any change in his or her health risk status.

Please direct questions or concerns to one or all of the following:

Scott Argraves, Argraves@musc.edu, 843-792-5482 (ofc), 843-697-5328 (cell), 843-557-0539 (home).

Sandy Klatt, klatts@musc.edu, 843-792-5483 (ofc), 843-810-8883(cell), 843-559-3408(home).

Cindy Gittinger, gittinck@musc.edu, 843-792-6236(ofc), 843-224-1503(cell), 843-881-2921(home).

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