



Biosafety Level 2 Standard Safety Requirements

Responsible Executive: Sr. VP/Provost

Responsible Office: EH&S Committee

Effective: December 18, 2015

Revised:

PURPOSE

The purpose of this policy is to outline safety procedures followed when working with Biosafety Level 2 (BSL-2) microorganisms. It should serve as a comprehensive source of procedures to be followed to ensure a safe working environment while working with these organisms.

SCOPE

The procedures established in this policy are to be followed by all individuals working with BSL-2 cultures in Wilkes University laboratories.

DEFINITIONS

Biosafety Level 2: “Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low...Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials.” (*Biosafety in Microbiological and Biomedical Laboratories*, 5th edition. U.S. Department of Health and Human Services, HHS Publication No. (CDC)21-1112.)

BSL-2 Laboratory: Any teaching or research laboratory on the Wilkes University campus in which manipulation of BSL-2 agents is conducted. At initiation of these policies, that is limited to Room 325 in the Cohen Science Center.

IBC: Institutional Biosafety Committee.

BMBL: *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition. U.S. Department of Health and Human Services, HHS Publication No. (CDC)21-1112. All policies herein adopted, and any future revision of these policies, must be in compliance BMBL guidelines.

BSC: Biological safety cabinet.

POLICY & PROCESS

Personnel:

- All persons working in or having access to CSC 325 are instructed on the hazards associated with working with BSL-2 agents. All persons conducting work receive instruction on the specific hazards associated with their work and the specific safety equipment, practices and behaviors required during the course of their work and use of the facilities.

- All personnel in the laboratory are trained in Biosafety Level 2 safety practices and will have access to BMBL.
- All laboratory personnel have access to IBC-approved project information and project-specific or microbe-specific protocols.
- All personnel are responsible for understanding the various risks for the agents with which they work and their proficiency in practices and techniques.
- Access to any laboratories working with Biosafety Level 2 cultures, while such work is being conducted, is limited to trained employees and students.

Specimens and Specimen Handling

- BSL-2 materials handled in CSC 325 are limited to previously purified bacterial cultures received on slants or sealed plates. **Specifically, the laboratory does not work with human materials, bloodborne pathogens or human viruses.**
- Liquid cultures are limited to a volume no greater than 3 ml, in screw-top culture tubes.
- All centrifugation of cultures is conducted in standard microcentrifuge tubes in a sealed rotor. Supernatants are removed by aspiration in a level II biological safety cabinet.

Equipment

- Available in the laboratory
 - Hand washing sinks
 - Biological safety cabinet
 - Emergency eyewash
- Available to all personnel
 - Personal Protective Equipment (PPE)
 - Face protection such as goggles, glasses, or disposable face shields
 - Disposable gloves
 - Mechanical pipetting devices

Materials/Reagents

- Biohazard safety signs, stickers, and trash receptacles
- Laboratory disinfectant, such as CiDecon
- Hand soap and absorbent disposable toweling
- Household bleach diluted fresh 1:10 to achieve 5% sodium hypochlorite
- 70% ethanol for use when bleach will be corrosive

Labeling and identification

- Biohazard safety signs are posted on the entrance to all laboratories where BSL-2 agents are handled, used or stored.
- Biohazard stickers are placed on all equipment that is used to manipulate or store BSL-2 agents, including but not limited to refrigerators, freezers and incubators.

Standard Precautions

- Standard precautions are practiced at all times in the laboratory when BSL-2 agents are handled.
- Hand washing is central to the biosafety goals of the laboratory and contributes to the health of all personnel. Hands are washed thoroughly with soap before leaving the laboratory.
- Loose hair is pulled back, away from the face and potentially contaminated surfaces.
- Shoes are closed-toe with nonslip soles.
- Clothing covers legs and arms to limit exposure to splash contamination. Laboratory coats are preferred and, if used, must be disposable and left in the laboratory.

- No eating, drinking, smoking, or application of makeup, adjustment of contact lenses, or any direct touching of eyes and other mucous membranes is permitted when any microbial agents are handled.
- All personnel strive to avoid the generation of aerosols when handling specimens or other potentially infectious materials.
 - Special care is used when working with Bunsen burners, so as not aerosolize pathogens with the flame; closed electronic incinerators are preferred over burners.
 - Centrifugation and vortexing of cultures occurs only in closed-capped microcentrifuge tubes that are allowed to settle at least 5 minutes prior to opening them; tubes are opened within a biological safety cabinet.
 - Screw-capped tubes are used for all handling of liquid cultures of BSL-2 agents in which aerosols may potentially be generated, including centrifugation, freezing and thawing of microbes. Snap cap tubes can be used when working with noninfectious lysates of these cultures.
 - Vigorous pipetting creates a risk for aerosolization and is performed in a BSC.
- All centrifugation of BSL-2 cultures should be done in a microcentrifuge with a sealed rotor.
- Mouth pipetting is prohibited in all Wilkes University laboratories.
- Microbial stocks are stored in freezers in locked rooms, and an inventory is maintained by the director of the microbiology laboratories, who also documents all transfers and use.
- Experiments are pre-planned within the context of the biohazards and other hazards that are imparted by agents and chemicals required for the experiment.
- No windows are opened contiguous to the laboratory.
- Wilkes University maintains an integrated pest management program to prevent mechanical vector transmission of BSL-2 agents out of the laboratory.

Preparation for Experiments

- Countertops are cleaned with disinfectant (e.g., CiDecon) with paper toweling prior to conducting any work in the microbiology laboratory.
- All necessary tools, including loops and pipettors, are collected before beginning work and returned to their place when work is completed.
- Disinfection of countertops is repeated upon completion of work.

Waste Disposal

- All waste generated in the laboratory, including cultures and any material coming in contact with cultures, whether or not the agents are BSL-2, is sterilized by autoclaving prior to disposal.
- Appropriately labeled and lined biohazard containers are provided in the laboratory.
- Used microcentrifuge tubes, pipette tips and disposable loops are initially collected at the benchtop in stainless beakers; these are autoclaved when filled.

EXCEPTIONS

NONE

FORMS

Biosafety Level 2 Standard Safety Requirements Acknowledgement (appended)

CROSS REFERENCE

Instructor Safety Requirement for Science Laboratories and Art Studios.

RESPONSIBILITY

The Principal Investigator/Faculty Member is responsible for ensuring that all members of the laboratory are familiar with safe research practices. The Environmental Health and Safety Committee provides guidance, information, training, and review of biological safety programs as needed.


RESOURCES

List of BSL-2 agents currently used in Wilkes University teaching laboratories.

Burkholderia cepacia
Chromobacterium violaceum
Klebsiella oxytoca
Klebsiella pneumoniae
Morganella morganii
Plesiomonas shigelloides
Proteus mirabilis
Proteus vulgaris

Pseudomonas aeruginosa
Salmonella enterica
Shigella sonnei
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

Approved By:

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12/18/2015 | 9:47 AM ET

Anne Skleder

Date

Senior Vice President/Provost

