

Microbiology Biosafety Manual



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I. Authority for Microbiology Lab and Prep Room Regulations

Black Hawk College Microbiology Laboratories and Prep Rooms will follow the guidelines posted by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. These guidelines describe acceptable biosafety practices in biomedical and microbiological laboratories and can be found at:

<http://www.cdc.gov/OD/ohs/biosfty/bmb15/bmb15toc.htm>

II. Definition of BSL1 and BSL2

Biosafety Level 1 is suitable for work involving microbiological agents not known to consistently cause disease in healthy adults, and present minimal potential hazard to laboratory personnel and the environment. BSL1 laboratories are not necessarily separated from the general traffic of the building. Work can be done on open bench tops using standard microbiological practices. Laboratory personnel must have specific training in the procedures used.

Biosafety Level 2 is suitable for work involving microbiological agents that pose moderate hazards to personnel and the environment and low community risk for infection. Laboratory personnel must have specific training in handling pathogenic and infectious agents. Access to the laboratory is restricted when work is being conducted and rooms are marked with BIOHAZARD sign.

Quad City Campus prep room 207, EC Room B212, and lab rooms QC 206, QC 207 and EC 216 are multipurpose rooms in which microbiology courses are held and prepared. These rooms use both BSL-1 and BSL-2 microorganisms. The following manual outlines BSL2 precautions. Whenever a BSL2 agent is in use, biohazard signs will be posted on the doors and the entire room will follow BSL2 practices. BSL2 precautions will be followed unless the instructor or laboratory technician has verified that only BSL1 organisms are present in the laboratory room in use.

In the case of only BSL1 organisms present and in use, the instructor or laboratory technician may choose to make the following substitutions to the precautions listed below: a. For BSL1 cultures glove use is optional for standard lab procedures if proper hand hygiene is used. Gloves are required when cuts are present on the hands or when staining microorganisms. b. For BSL1 liquid cultures safety goggles or safety glasses are required but masks are optional.

III. Regulations

A. Access, Training, and Responsibilities

1. QC prep room 207 and EC B212 access is limited to individuals involved directly in media prep, clean up, lab prep and research. QC classrooms 206/207 and EC B216 are also accessed by students under supervision of instructor.
2. The labs and prep room doors will be closed when microorganisms are in use.

3. Faculty, staff and work study students are required to read, understand, and follow these regulations before working in microbiology labs. There is a paper to sign after reading this document to provide proof of getting this document. This paper will be kept on file by the science lab technician.
4. Faculty, staff and work study students working in classrooms QC 206/207 and EC B216 and prep rooms QC 207 and EC B212 will receive training from their instructor/supervisor concerning use of the equipment.
5. The instructor/supervisor will train faculty, staff and work study students on aseptic techniques appropriate for handling pathogenic agents. This will include the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Students working with microorganism in a laboratory course will be instructed of proper techniques by the instructor and will complete the classroom “Microbiology Laboratory Safety Rules and Procedures” safety sheet which is kept on file with the Science Laboratory Technician.
6. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
7. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
8. Any faculty, staff or work study students found in violation of the regulations may have their access to these rooms terminated.
9. Microbial cultures and bio hazardous waste will be secured or removed before the room is used for other purposes.

B. Apparel

1. Persons entering classrooms QC 206/207 and EC B216 and prep rooms QC 207 and EC B212 will be required to wear closed-toe shoes and have long hair tied back.
2. Persons working in QC prep room 207 and EC B212 and classrooms QC206/207 and EC B216 must wear lab coats at all times. The coats must be worn properly which includes having sleeves cover street clothes and buttoned from top to bottom. This protective clothing is removed and left in laboratory before leaving for non-laboratory areas (e.g., restrooms, break room, or offices).
3. Gloves are worn when handling microorganisms or hazardous chemicals. Gloves are disposed of when contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Gloves are placed in a biohazard bag and autoclaved prior to disposal. Disposable gloves are not washed, reused, or used for touching clean surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Hands are washed following removal of gloves.

4. Persons working in prep rooms QC 207 and EC B212 and classrooms QC 206/207 and EC B216 must wear appropriate eye protection (safety goggles or safety glasses) for normal lab procedures involving liquid cultures that do not generate a splash hazard. (e.g., proper pipetting spread plates, etc.). Safety goggles and masks are worn when performing procedures that may create a splash hazard.

5. When working in a biosafety cabinet, only lab coats and gloves are needed for personal protection.

C. Standard Microbiological Practices

1. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the lab. Food for human consumption is never stored in the lab.

2. An orange biohazard sign must be posted on the entrance to the laboratory when etiologic agents (disease causing) are in use. Information to be posted includes:

BSL2 level organisms in use Lab technician's name and phone number

Biohazard symbol PPE required in use in the lab room

Procedures for exiting the lab

3. Persons wash their hands upon entering the lab, after they finish working in the lab, after removing gloves, and before leaving the laboratory.

4. Work surfaces are decontaminated prior to beginning any work in these rooms, on completion of work or at the end of the day with 10% bleach solution. Any spill or splash of viable material should be decontaminated with 25% bleach solution.

5. All procedures are performed carefully to minimize the creation of splashes or aerosols.

6. Mouth pipetting is prohibited: mechanical pipetting devices are used.

7. All cultures, swabs, and waste containers are decontaminated before disposal by autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container for transport from the laboratory.

8. The area for culturing should be separate from the area taking notes. If a pull-out shelf is available, this should be used for note taking.

9. All culture tubes will be labeled with genus and species.

D. Special Practices

1. Access to the laboratory is limited or restricted by the instructor when work with infectious agents is in progress. Persons who are at increased risk of acquiring infection – e.g., those who are immunocompromised or immunosuppressed – or for whom infection may have serious consequences, should consult with their physician to determine the appropriate level of participation in the lab.

E. Transfer of Materials

1. Cultures, and/or potentially infectious wastes are placed in a container that prevents leakage during collection, handling, processing, storage, and transport.

F. Disposal of Materials and Decontamination

1. Laboratory equipment and work surfaces should be decontaminated with 10% bleach on a routine basis and after work with infectious materials is finished. Overt spills, splashes, or other contamination by infectious materials should be decontaminated with 25% bleach.

2. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the instructor. If needed, medical attention, surveillance, and treatment are provided as appropriate and written records are maintained.

**** Spills should be covered with paper towels and the disinfectant poured around and over the spill. Saturate the area with disinfectant and leave undisturbed for 15 to 30 minutes. After the time, place all paper towels and other affected items in biohazard bag to be autoclaved. Then spray down area with disinfectant one more time.**

3. Broken glassware that does not contain live cultures should be swept up with the broom and dust pan and discarded in the glass disposal box.

4. Broken glassware that contains live cultures should be saturated with bleach solution. After 15 minutes, the debris should be swept up into an autoclave bin using paper towels. After being autoclaved, the glassware can go into the glass disposal box and the paper towels into the regular trash.

5. Any items that get contaminated by a live culture will be autoclaved. This includes any personal item of the students, and clothes.

6. Autoclave Requirements

- a. 121°C at 15 psi for 15 min.
- b. Loaded in a manner that ensures that steam can penetrate packages and test tubes
- c. Log book kept with date, time, items autoclaved and initials
- d. Indicator tape will be placed on every load to verify sterility.
- e. Monthly indicator ran and documented.

G. Hygiene and Housekeeping

1. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and bleach used to decontaminate the work surfaces.

2. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs used in laboratory work should be covered with a nonporous material that can be easily decontaminated.

3. Safety Data Sheets (SDS) are located in prep room QC 207 and EC B216

4. First aid kit is located in QC conference room and B Lounge area EC.

5. Eye wash stations are located:

QC room 206 at every sink and prep room

QC room 207 at the first and third sink and prep room

EC B212 next to fume hood.

6. The shower is located in QC room 214 and EC B212.

7. The fire extinguisher is located:

QC room 206 front of the room to the right and prep room

QC room 207 to the rear of the room on the left and prep room

QC several hallway locations.

EC room B 216 to the left of the microscope cabinet, and 1 to the left of the sink.

8. The fire blanket is located:

QC room 206 front of the room to the right

QC room 207 to the rear of the room on the left

EC room B210 next to counter by eyewash/shower

EC room B216 next to counter before B212

EC room 219 left of entrance door and another before B212

9. Goggle station with UV sterilization is located:

QC room 206 front of the room to the right.

QC room 207 rear of the room to the left

EC room B216 right side of the room

10. Disinfectant spray bottles are located: near the sinks in QC room 206 and 207 and EC sink or side counter.

11. Lab coats are hanging in every room for use. They are laundered at the end of each semester or immediately after a spill or splash.

H. Exposure to Infectious Materials

What to do if you are exposed to potentially infectious material to the eyes, nose, mouth, or broken skin?

1. Immediately flood the exposed area with water
 - a. In case of exposure to eyes flush for 15 minutes
2. Clean wound with soap and water or use a skin disinfectant
3. Report immediately to your Instructor/Supervisor and an Incident Report must be

filled out, and sent to Risk Management (QC) within 24 hours. Any follow-up medical care should also be recorded on the incident form. The Incident Form is available in myBlackhawk under Finance, Risk Management, Accident/Incident Form.

4. **Seek immediate medical attention and you may need to receive post-exposure prophylaxis called PEP in accordance with CDC guidelines**
5. And/or call the National Clinicians PEP Hotline at 1(888)448-4911

IV. List of Microbiological Cultures used in BHC Labs

BSL-1

Alcaligenes faecalis

Mycobacterium smegmatis

Aquasprillum serpens

Pseudomonas fluorescens

Aspergillus niger

Saccharomyces cerevisiae

Bacillus cereus

Serratia marcescens

Bacillus stearothermophilus

Staphylococcus epidermidis

Bacillus subtilis

Streptococcus mutans

Citrobacter freundii

Streptococcus salivarius

Corybacterium xerosis

Enterobacter aerogenes

Escheria coli

Lactococcus lactis

Micrococcus luteus

BSL-2

Corynebacterium diphtheriae

Salmonella typhi

Enterococcus faecalis

Shigella flexneri

Klebsiella pneumonia

Staphylococcus aureus

Proteus vulgaris

Streptococcus pneumoniae

Pseudomonas aeruginosa

V. MICROBIOLOGY LABORATORY SAFETY RULES AND PROCEDURES

The following rules are in addition to the general Black Hawk College Biology Laboratory Safety Rules and Procedures. The lab exercises in this course involve the use of living organisms. Although the microorganisms we use are not considered to be highly virulent, **all microorganisms should be treated as potential pathogens** (organisms capable of causing disease). The following rules must be observed at all times to prevent accidental injury to and infection of yourself and others and to minimize contamination of the lab environment.

1. Clean your work area with labeled disinfectant at the beginning and end of each lab period.
2. Wash your hands with soap and dry with paper towels at the beginning and end of each lab period.
3. Never place books, backpacks, purses etc. on bench tops. Always place these in the assigned areas. Notes and pens or pencils should be put away when working with microorganisms.
4. Electronic devices should not be brought into the lab. This includes laptops, tablets, iPods, MP3 players, cell phones, calculators, etc.
5. **Any item** that gets contaminated will be autoclaved before it is returned to the student.
6. Students working in the lab must be under instructor supervision.
7. Clothing and Protective Equipment Requirements:
 - A. A **lab coat** is to be worn all times while working in the lab to prevent contamination and/or accidental staining of your clothing. The lab coat must be fully buttoned.
 - B. **Closed-toed shoes** (no sandals) are to be worn in the lab.
 - C. **Long hair must be tied back** to prevent exposure to flame and contamination of cultures. No dangling jewelry is to be worn.
 - D. **Gloves** are to be worn when staining microbes and handling microorganisms.
 - E. Appropriate **protective eyewear** is to be worn during wet labs. **Goggles** are to be worn when working with splash hazards.
 - F. Masks are to be worn when working with splash hazards.
8. Keep your hands and other objects (pencils, etc.) away from your mouth and eyes.
 - A. Eating and drinking are prohibited in the lab at all times (includes gum, cough drops, and candy).
 - B. Do not apply cosmetics, lip balm, lotion, etc. in the lab.
 - C. Do not insert or adjust contact lenses.
 - D. Never pipet by mouth, use a mechanical device.
9. Do not remove media, equipment, or bacterial cultures from the laboratory. It is prohibited.
10. Carry cultures in a test tube rack when moving around the lab, or while at bench top.
11. Reusable inoculation tools must be flamed before placing them on the bench top. Disposable inoculation tools must be placed directly in the designated disposal container and must not touch the bench top.
12. Report any accidental spills, cuts or burns immediately to your instructor.
For spills of cultures: Cover with paper towels and saturate them with spill disinfectant solution. After 15 minutes, dispose of towels and broken items as indicated by instructor.
13. Place all cultures and materials in the proper disposal areas at the end of lab.
14. Persons who are immune-compromised (including those who are pregnant or may become pregnant, and those living with an immune-compromised person) should consult with their physician to determine the appropriate level of participation in the lab. Please have physician write a note stating the concerns and alternative accommodations that may be made.

I, the undersigned, state that I have read and understand the above Microbiology Laboratory Safety Rules and Procedures and that I have been notified of my responsibility to observe and follow all safety measures.

Print Name: _____ Date: _____

Signature: _____

Course: _____

The following rules and procedures must be observed at all times to prevent accidental injury and contamination of yourself and others and to maximize the safety of the laboratory environment.

1. When you are in the laboratory, be alert and think about what you are doing. Follow instructions carefully, and ask your instructor if you have questions before proceeding.
2. Never place books, backpacks, purses etc. on bench tops. Always place these in the assigned areas. Notes and pens or pencils should be put away when working with microorganisms.
3. Clothing and Protective Equipment Requirements:
 - A. Appropriate protective eyewear is required for wet lab activities. Goggles are required for splash hazards.
 - B. To prevent contamination, wear gloves, lab coats and other protective apparel when instructed. Wash your hands before leaving the laboratory.
 - C. Closed-toe shoes are required in the laboratory.
 - D. Long hair must be tied back to prevent contamination.
4. Keep your hands and other objects (pencils, etc.) away from your mouth and eyes.
 - A. Do not eat, drink, smoke, or chew gum in the lab.
 - B. Do not insert or adjust contact lenses while in the laboratory.
 - C. Do not apply cosmetics in the lab.
5. Learn the location and correct operation of all safety equipment: fire extinguishers, fire blankets, eye wash stations, first aid kits, etc.
6. Inform the lab instructor of any health conditions (pregnancy, allergies, passing out, contact lenses, etc.).
7. Use laboratory equipment properly and according to instruction.
8. Never aim the opening of a test tube or flask at yourself or others.
9. Do not insert transfer pipettes into reagent bottles.
10. When gathering materials, read label carefully, take only what you need, and do not return any unused portion to the original container.
11. Do not use unlabeled chemicals.
12. Do not use your fingers as a stopper for a tube or bottle when shaking to mix contents.
13. If you accidentally ingest a chemical or get it in your eyes or on your skin, immediately notify your instructor so proper measures can be taken. Report any accident, however minor, to your lab instructor immediately.
14. Do not taste any chemical or biological agent. When testing odors, fan the vapor to your nose with your hand. Only test odors when instructed.
15. Use extra caution if dissecting, since you are using sharp tools and you might be subjected to toxic chemical vapors.
16. Properly dispose of all wastes. Unless otherwise stated, solids should be placed in the waste containers and liquids should be poured down the sink.
17. Place contaminated materials, items soiled with blood or other bodily fluids into biohazard containers designated by the instructor. Place bacterial cultures into the red autoclave bags.
18. Loose hair and clothes are prohibited around open flames. Do not leave heat sources unattended and always keep all flammable liquids away from an open flame. Be mindful of hot glassware and always use appropriate devices for handling it.
19. Use caution when handling glassware. If glassware breaks, report it immediately and your instructor will help you clean it up safely and dispose of it in a glass disposal receptacle.
20. If you spill a chemical or microbial culture, report it immediately and your instructor will help you clean it up safely.
21. Students working in the lab must be under instructor supervision.
22. Never remove equipment, chemicals, biohazards, or specimens from the laboratory.
23. Before leaving lab, check that your work area is clean and in proper order. If you use a microscope make sure you store it away properly according to your instructor's instructions.

When working with microbial cultures, follow the Black Hawk College *Microbiology Laboratory Safety Rules and Procedures*.

I, the undersigned, state that I have read and understand the above Biology Laboratory Safety Rules and Procedures and that I have been notified of my responsibility to observe and follow all safety measures.

Print Name: _____ Date: _____

Signature: _____

Course: _____

VII.



**ROOM ACCESS IS RESTRICTED TO
AUTHORIZED PERSONNEL ONLY!**

BSL 2 Organisms may be in use.

**All personnel must wear long pants, closed-toed shoes,
lab coats, goggles and gloves. Long hair must be tied
back.**

Prior to EXIT: Remove lab coats, goggles and gloves.

Wash Hands thoroughly.

QC Linda Peterson Science Laboratory Technician 796-5244

EC Chris Rebout Science Laboratory Technician 854-1854

VIII. Microbiology Biosafety Manual Training Record

I have received a copy of and have read and understand the regulations and procedures used in the Black Hawk College Microbiology Biosafety Manual. I have been trained in the use of the equipment available and aseptic techniques needed to work with the bacterial cultures used in the laboratory.

Signature

Date