

BIOSAFETY MANUAL FOR BIOSAFETY LEVEL 2 (BSL-2) LABS

COLLIN COLLEGE

Preston Ridge Campus

MICROBIOLOGY

Room LH201 | Lawler Hall

Date of Last Revision: July 29, 2015

This Biosafety Manual has been prepared for the Collin College laboratories located at the Preston Ridge Campus. Room numbers, laboratory descriptions and locations of safety related equipment and procedures outlined in this manual are specific to the Preston Ridge Campus laboratory facilities. This manual has been adapted from the 2012 Appendix to the Guidelines for Biosafety in Teaching Laboratories published by the American Society for Microbiology (ASM).

I. Authority for Microbiology Lab and Prep Area Regulations

The Preston Ridge Campus microbiology labs will follow the guidelines posted by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). These guidelines describe acceptable biosafety practices in biomedical and microbiological laboratories and can be found at: <http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>.

Rooms LH205 and LH208 at the Preston Ridge Campus are multipurpose laboratory rooms (prep areas). Biosafety Level 1 (BSL-1) precautions will be followed during routine media prep, autoclaving, and subculturing.

Any time an agent requiring Biosafety Level 2 (BSL-2) containment is in use, biohazard signs will be posted on the doors and the entire room will follow BSL-2 practices.

Room LH201 is the only room on the Preston Ridge Campus where work with BSL-2 agents is permitted. Generally, work with all cultures for the microbiology program at PRC should be performed in Room LH201.

Summary of Recommended Biosafety Levels for Infectious Agents

Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices (SMP)	None required	Open bench-top sink required
2	Associated with human disease, hazard = autoinoculation, ingestion, mucous membrane exposure	BSL-1 practices (SMP) plus: ⇒ Limited access ⇒ Biohazard warning signs ⇒ Sharps precautions ⇒ Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials as well as PPE such as laboratory coats, gloves, face protection as needed	BSL-1 plus ensure that an autoclave is available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	BSL-2 practice plus: ⇒ Controlled access ⇒ Decontamination of all waste ⇒ Decontamination of lab clothes before laundering	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents as well as PPE such as protective lab clothing, gloves and respiratory protection, as needed	BSL-2 plus: ⇒ Physical separation from access corridors ⇒ Self-closing, double door access ⇒ Exhaust air not recirculated ⇒ Negative airflow into laboratory

BSL-3 and BSL-4 are not permitted at Collin College

The Preston Ridge Campus Microbiology Labs are limited to working with organisms requiring up to Biosafety Level 2 containment only.

Organisms requiring Biosafety Level 3 or Biosafety Level 4 containment are not permitted to be purchased, stored or otherwise acquired by Collin College.

Standard Microbiological Practices, as defined by the *CDC's Biosafety in Microbiological and Biomedical laboratories, 5th Edition* alongside our current microbiology laboratory practices at the Preston Ridge Campus:

<i>CDC Recommendations for Standard Microbiological Practices</i>	<i>Current Practices at the Preston Ridge Campus</i>
1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.	1. All science laboratory classrooms and prep areas are secure areas with access controlled by keypad locks. Access codes are only provided to authorized faculty and staff. Real or perceived breaches in security are reported to the Campus Police Department.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.	2. Hand washing is required by all students faculty and staff. WASH your hands with soap and warm water before leaving the lab and/or after removing contaminated gloves. Do not wear personal protective equipment (gloves, lab coats, etc.) out of the laboratory area unless you are transporting hazardous materials or wastes.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.	3. DO NOT eat, drink, smoke/use tobacco products, store food, or apply cosmetics in the laboratory. Faculty and staff are permitted to have drinks in CLOSED containers at PREP AREA computer workstations only. Food and drink are never permitted in any of the laboratory classrooms.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.	4. MOUTH PIPETTING is strictly prohibited; use mechanical pipetting devices.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include: a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving. d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.	5. See Waste Management Plan and Biological Waste Table for current practices. BROKEN GLASS: Do not handle broken glassware with bare hands. Dispose of all cracked or broken glassware in a puncture-resistant container – NOT the regular trash. Discuss with instructor what was broken and where to discard it. Discard broken glassware contaminated with hazardous chemicals in an appropriately labeled container. See your instructor or a member of the lab staff if a hazardous waste/broken glass container is needed. Lubricate glass pieces before inserting into corks, stoppers, etc. To prevent glass breakage, insert glass with a twisting motion. Medical Waste Containers: Red plastic medical waste containers are to be used for any glass or sharp object that has come in contact with potentially biohazard material such as bacteria, blood, urine, saliva and/or other human or animal fluids.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.	Preparing cultures in volumes that exceed the capacity of a 13 mm OD x 100 mm test tube (approximately 8 milliliters) are not permitted. The added use of a face shield is required when work involving anticipated aerosols or splashes must be performed.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.	7. CLEAN and disinfect work surfaces at the beginning and end of every lab period. Keep extra books, bags and clothing in designated places so your work area is uncluttered.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport. a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport. b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.	9. See Waste Management Plan for a full description of our biological waste management practices. See the Biological Waste Table for biological waste stream profiles and current disposal methods. 1) Biohazard bags (from step cans): bags are sterilized on-site in accordance with the provisions of 25 TAC §1.136 and disposed of as solid waste. 2) Red Sharps Container: regulated medical waste is disposed of through a contracted vendor. 3) Pipette Tray (for GLASS serological pipettes only): glass pipettes are disinfected and reused. 4) White Buckets (for tubes): tubes and caps are autoclaved and reused. Sterilized liquid media is disposed of via sanitary sewer. Sanitized solid media is collected in a container, labeled in accordance with the provisions of 25 TAC §1.136 and disposed of as solid waste. 5) Regular Trash: items thrown into the regular trash cans are for uncontaminated waste only. This waste goes to the dumpsters. 6) Broken Glass Bucket: uncontaminated broken glass is disposed of in a puncture resistant container. 7) Biohazard + Hazardous Waste: Tubes are first disinfected to remove biohazard. Remaining waste is treated and disposed of as Hazardous Waste. 8) Animal wastes (carcasses, tissues, bones, skin) are collected for incineration through a contracted vendor.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.	9. A sign incorporating the universal biohazard symbol is posted at the entrance of the instructional laboratory.
10. An effective integrated pest management program is required.	10. IPM is under the authority of Facilities/Plant Operations.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.	11. All laboratory personnel are trained annually in the following areas: <ul style="list-style-type: none"> • RCRA Hazardous Waste Generator Training • DOT Hazmat Training • Biosafety Training • Orientation to Laboratory Safety • Safety Showers and Eye Washes in the Laboratory • Flammables and Explosives in the Laboratory • The Formaldehyde Standard • Electrical Safety in the Laboratory • Laboratory Ergonomics • Using MSDSs in the Laboratory • Laboratory Hoods • Preventing Contamination in the Laboratory • Safe Handling of Laboratory Glassware • Planning for Laboratory Emergencies • The Basics of First Aid

II. Regulations

A. Access, Training and Responsibilities

1. Access to LH201 is limited to individuals involved directly in media prep, clean-up, laboratory prep, faculty facilitated research (CASMNS), and building maintenance. CASMNS students participating in research related projects must complete additional safety training prior to working in LH201.
2. The lab and prep room doors will be closed when a BSL-2 agent is in use.
3. All staff and students are required to read, understand, and follow these regulations before working in LH201.
4. All staff and students working in LH201 will receive training from their instructor or supervisor concerning use of the equipment.
5. The Lab Manager will train staff and faculty will train 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.
6. Laboratory staff members receive annual updates or additional training as necessary for procedural or guideline changes. Biosafety Training is performed and documented annually for all microbiology faculty and staff. Students must demonstrate proficiency in BSL-1 laboratory safety techniques prior to being permitted to work with BSL-2 organisms. Faculty members are responsible for training and testing their students in all areas of laboratory safety and biosafety practices.
7. Laboratory personnel and students are advised of special hazards and are required to read and follow instructions on practices and procedures.
8. Any staff or students found in violation of the regulations may have their access to the laboratory areas terminated.
9. The Laboratory Manager and teaching faculty are responsible for seeing that the consequences of student or staff actions are rectified, including correction of damages and violations and take-down of experiments.

B. Apparel

1. Personnel entering room LH205 or LH201 will be required to wear closed-toe shoes and have long hair tied back.

2. Personnel working in LH201 at BSL-2 must wear lab coats at all times when cultures are in use. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., restroom, cafeteria, library, or administrative offices).
3. Gloves must be 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 to be washed following the removal of gloves.
4. During work with any live cultures, chemical splash goggles must be worn for normal lab procedures involving liquid cultures that do not generate a splash hazard (e.g., proper pipetting, spread plates, etc.). Chemical splash goggles and face shields are to be worn when performing procedures that may create a splash hazard. At a minimum, chemical splash goggles must be worn when working with bacterial cultures (for both BSL-1 and BSL-2 organisms).
5. Lab coats should never be shared and must always stay in the laboratory. Students should store their lab coat in a ziptop bag that is then kept in a designated space in the laboratory. At the end of the semester, items kept in the lab must be decontaminated before students are allowed to take them home. Ziptop bags are available from a member of the lab staff. Disposable lab coats must be disposed of in a red bag and autoclaved.
6. Shorts and sleeveless shirts are never recommended for the laboratory setting.

C. Standard Laboratory Practices

1. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the lab. Cell phones, tablets, and laptops should not be used in LH201. Food and drink for human consumption is never to be stored the lab.
2. An orange biohazard sign must be posted on the entrance to the laboratory when biological agents are in use. Information to be posted includes the agent(s) in use, biohazard symbol, biosafety level 2, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
3. Persons must 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 must be decontaminated prior to beginning any work in LH201, on completion of work or at the end of the day with 10% bleach solution, prepared Lysol solution or 70% ethanol solution. Any spill or splash of viable material should be

decontaminated with 25% bleach or Lysol concentrate. NOTE: Materials that have been soaked in bleach should never be autoclaved.

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. Needles and syringes may be used for reconstituting reagents. After use, these materials are placed in a puncture-proof red sharps container. Do not recap needles.

8. All cultures, swabs, and waste containers must be 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. All red sharps containers are transported off site for decontamination and final disposal. Full red sharps containers should be brought to Room J223F for final packaging prior to shipping off site.

9. Microincinerators are recommended for the teaching laboratory due to the dangers posed by open flames in the laboratory. The microincinerator reaches an internal temperature of 1500°F, easily sterilizing loops and needles in just a few seconds. Microincinerators also eliminate the possibility of cultures creating splatter or aerosolizing during the sterilization process.

Heat-fixing can be accomplished by holding the slide over the opening of the microincinerator. Microincinerators are used in the LH205 and LH201 laboratories instead of the traditional Bunsen Burners. There are two incinerators at each lab bench. DO NOT leave loops or needles unattended in the incinerator at any time. Always turn OFF and UNPLUG the incinerator at the completion of the lab.

D. Special Practices

1. Laboratory Access

Access to the laboratory is limited or restricted by the Laboratory Manager 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. Employees that have a medical condition that might put them at a greater risk for infection should consult with their supervisor prior to conducting any work in the BSL-2 laboratory. Students that have a medical condition that might put them at a greater risk for infection should consult with their instructor prior to conducting any work in the BSL-2 laboratory.

2. Laminar Flow Hood / Clean Bench

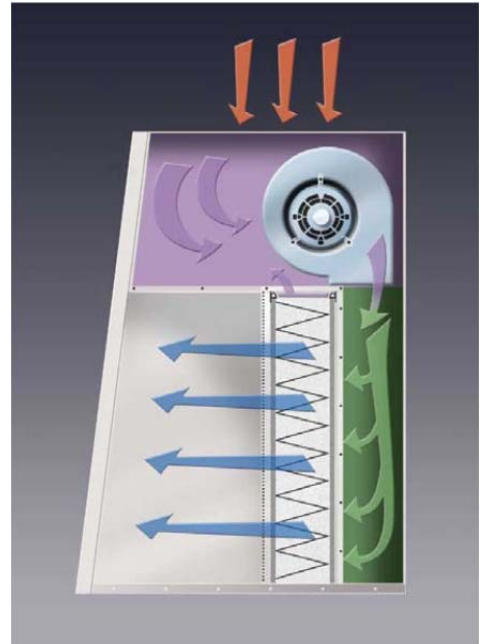
Room LH205 is equipped with a Labconco Clean Bench that is to be used for the preparation of microbiological media and other tasks not involving biological agents or hazardous chemicals. As pictured, the motor/blower assembly pulls air through the pre-filters on the top of the bench, and flows through the HEPA filter, which then flows horizontally through the work area.

Because air from the work area is dispersed directly into the laboratory, the Purifier Clean Bench should never be used in conjunction with biohazardous material.

The Clean Bench in LH205 is recertified annually. The pre-filters, located at the top of the unit should be replaced every 12 weeks.

Use of the UV light is not permitted since this unit is located in a shared prep area, making it difficult to restrict access when the UV light is in use.

Read the User's Manual located in LH205 prior to operating the Clean Bench. Report any problems with operation to the Lab Manager.



Follow the weekly, monthly and quarterly routine maintenance schedule posted on the side of the unit.

3. Biological Safety Cabinet

The Preston Ridge campus is not equipped with a Biological Safety Cabinet. Procedures that create the potential for creating infectious aerosols or splashes should be avoided. Preparing cultures in volumes that exceed the capacity of an 18 mm OD x 150 mm test tube (approximately 35 milliliters) are not permitted. The added use of a face shield is required when work involving anticipated aerosols or splashes must be performed.

E. Transfer of Materials

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

2. If cultures or biological wastes must be transferred between buildings, materials must be carried within secondary containment while en route. Always transport wastes on a cart.

F. Disposal of Materials and Decontamination

1. Laboratory equipment and work surfaces should be decontaminated with 10% bleach, prepared Lysol solution or 70% ethanol solution 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 or Lysol concentrate. Alcohol based disinfectants should never be used near an open flame or other heat source. NOTE: Materials that have been soaked in bleach should never be autoclaved.

2. Spills and accidents that result in obvious exposures to infectious materials are to be immediately reported to the Lab Manager. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

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 bucket. Broken glassware should never be handled with bare hands.

4. Broken glassware that contains live cultures should be saturated with Lysol concentrate. After 15 minutes, the debris should be carefully collected for disposal in a red sharps container. Small glass fragments should be picked up with forceps. Paper towels used for clean-up should be discarded in the autoclave step can for final sterilization and disposal.

5. The PRC Microbiology Labs adhere to the Texas Regulations on Medical Waste as outlined in 30 TAC 330, Subchapter Y. Autoclave efficacy testing is performed on a weekly basis.

6. All biological waste that is treated on site is sterilized by autoclaving in Room LH205. Each red bag is sterilized at a minimum of 121°C at 15 psi for 60 minutes. Each load of tubes to be decontaminated is sterilized at a minimum of 121°C at 15 psi for 60 minutes. Bagged and tubed waste is never to be sterilized in the same load. Run one bag per cycle and run tubes separate from bags.

Record Keeping and Autoclave Testing

The Texas Commission on Environmental Quality (TCEQ) regulates the handling and on-site treatment of all regulated medical waste (RMW). These waste streams include microbiology waste, bulk blood (volumes over 100 milliliters), sharps and pathological waste. Per TCEQ regulations, the following documentation must be kept for all RMW that is treated on-site. Collin College treats RMW by steam sterilization or chemical disinfection when steam sterilization is not practical.

A generator of more than 50 pounds per calendar month of medical waste that treats all or part of the wastes on-site and persons that treat medical wastes off-site shall maintain a written record that, at a minimum, contains the following information for each batch of waste treated:

- (A) the date of treatment;
- (B) the amount of waste treated;
- (C) the method/conditions of treatment;
- (D) the name (printed) and initials of the person(s) performing treatment; and
- (E) a written procedure for the operation and testing of any equipment used and a written procedure for the preparation of any chemicals used in treatment.

The operator shall demonstrate a minimum four log ten reduction (as defined in 25 TAC '1.132 (relating to Definitions) on routine performance testing using appropriate *Bacillus* species biological indicators (as defined in 25 TAC '1.132). The operator shall conduct testing at the following intervals:

- (I) for generators of more than 50 pounds but less than or equal to 100 pounds per month, testing shall be conducted at least once per month;
- (II) for generators of more than 100 pounds but less than or equal to 200 pounds per month, testing shall be conducted at least biweekly; and
- (III) for generators of more than 200 pounds per month and persons that treat medical wastes off-site, testing shall be conducted at least weekly.

The Preston Ridge Campus can generate over 150 pounds of autoclaved red bags alone in a single month. In order to assure validation testing compliance, autoclave testing is performed on a weekly basis with *Geobacillus stearothermophilus*, spore population 10^5 .

Instructions for the use of self-contained biological indicators:

(VWR Item# 95029-702)

<http://www.namsa.com/Portals/0/Documents/SCS-05-technicaldatasheet.pdf>

- 1) To be completed by full time staff member or Lab Manager: On Tuesday or Wednesday of each week, label one unused self-contained biological indicator (SCBI) with the date and waste load number. Record date of test, SCBI lot number, expiration date, waste load number and user name on Autoclave Test Log.

- 2) Place labeled SCBI inside the load to be tested. Do not test the autoclave on an empty load. The indicator should be positioned inside the autoclave bag or among the racks in the most interior part of the load. Secure the indicator to a strip of autoclave tape or a long glass rod in order to safely retrieve the capsule after sterilization.
- 3) Allow capsule to cool so it may be safely handled. Immediately following sterilization and cool down, seal the cap by pressing down firmly until the media ampoule is crushed. This activates the ampoule.
- 4) Place activated SCBI into a 55–60°C incubator for a minimum of 24 hours, max of 30 hours.
- 5) To be completed by a second staff member or lab manager: Examine for growth after 24 hours, record date/time, results (Color & Pass/Fail) and your name. Prolonged incubation may have adverse positive effects.
- 6) Interpreting Validation Results: The acid production associated with bacterial growth causes a shift in the color of the culture medium from purple to yellow.

Purple = Negative for growth; **Yellow** = Positive for growth*
- 7) Discard/Disposal: Autoclave all activated SCBIs for 45 minutes at 121°C prior to discard. Sterilized SCBIs may be disposed of in a red sharps container.
- 8) Store unused SCBIs in their original box, at room temperature. Do not freeze or refrigerate.

***ANY INSTANCES OF A FAILED VALIDATION TEST (YELLOW RESULT) MUST BE REPORTED TO THE LAB MANAGER IMMEDIATELY. ANY FUTURE USE OF THE AUTOCLAVE FOR WASTE TREATMENT PURPOSES MUST BE HALTED UNTIL THE UNIT CAN BE SERVICED AND RE-VALIDATED. ANY MEDIA PREP THAT IS PERFORMED BEFORE THE UNIT CAN BE INSPECTED MAY BE COMPROMISED.**

Biological Waste: Any waste that is potentially biohazardous, infectious or pathological
Biological waste includes:

- ⇒ human and animal blood, blood products, body fluids, tissue, and body parts;
- ⇒ cultures and stocks of infectious agents;
- ⇒ dissection waste from preserved or fresh specimens;
- ⇒ items such as gloves, paper towels and plastic that are soiled with infectious materials.

⇒ Biological waste may also include sharps materials (slides, scalpels, pipettes, etc.).

Packaging and Labeling Biological Waste for Disposal

Biohazard Cans/Autoclave Bags

Red biohazard bags inside of the Biohazard Cans are used for the collection of biological waste that is to be autoclaved. Sharp items (loops, swabs, slides, glass pipettes) should never be placed in an autoclave bag since these items can puncture the bag. Autoclave bags are used to collect contaminated disposable petri dishes, gloves, paper towels. Sharps of any kind are never permitted in an autoclave bag.

Biohazard Sharps Containers

The red sharps containers located throughout the laboratory classrooms and prep areas are to collect only the following items: glass slides and cover slips, contaminated swabs or disposable loops, broken glass that is contaminated with a biological or infectious agent, small glass bottles/vials of animal blood (10 milliliters or less. Full sharps containers are to be transported to J223F for collection.

Pathological Waste Containers (Located in J223F)

These 31 gallon collection boxes are reserved specifically for the following items: dissection waste (carcasses, skin, bone, tissue, etc.) from all preserved and fresh specimens. Dissection waste is to be placed into a red bag, sealed and then placed in the Pathological Waste Container for pick up and disposal. Path Waste Containers are disposed of by incineration through a contracted vendor. Microbiology labs do not generate dissection waste.

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. All bench top surfaces in LH205 and LH201 should be cleaned and sanitized on a daily basis.
2. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are to remain accessible for cleaning. Chairs used in laboratory work are covered with a nonporous material that can be easily decontaminated. All seating covers should be cleaned and sanitized on a weekly basis.
3. Safety Data Sheets (SDS) are located in LH201 on top of the incubator near the laboratory entrance.

4. First aid kits are located in LH201 at the rear of the room near the middle sink.
5. Eyewash station and safety shower are located in LH201 near the laboratory classroom door.
6. Fire extinguishers are mounted on the wall near the laboratory classroom door in LH201.
7. Refrigerators and Incubators should be cleaned and sanitized on a monthly basis with a freshly prepared 10% bleach solution.

H. Biological Spill Response

The following biological spill kit materials should be kept in each laboratory where work with microorganisms is conducted. Basic materials are: disinfectant (household bleach or Lysol I.C. Quaternary disinfectant), paper towels, nitrile gloves, autoclave bags, sharps container, and forceps to pick up broken glass.

For any biological spill, first notify others in the laboratory so they will not unknowingly spread the contamination. For biological or chemical spills of a large volume, evacuate the area and call for outside help.

Collin Police Department: 5555
City of Frisco Fire Department: 911

For small, common laboratory spills:

1. Wear gloves, lab coat and goggles. If splashing is likely, also wear face shield.
2. Use forceps to pick up broken glass and discard into a red sharps container. Do not pick up broken glass with gloved or bare hands.
3. Cover spilled material with paper towels.
4. Carefully **pour (do not spray)** diluted Lysol I.C. Quaternary disinfectant (from spray bottles or carboys) onto paper towels in sufficient quantity to ensure effective microbial inactivation.
5. Allow a **20-minute** contact period.
6. Pick up paper towels and dispose in biohazard waste container.
7. Re-wipe spill area with Lysol disinfectant. Place paper towels in biohazard container.
8. Mist area with Lysol disinfectant or a freshly prepared 10% bleach solution. Allow treated area to air dry. Keep area free from traffic until all surfaces have been properly disinfected, dried and spill materials cleaned up.

9. Place all contaminated materials, including any contaminated Personal Protective Equipment, into biohazard waste container and autoclave. DO NOT autoclave waste materials that have soaked in bleach solutions.

10. Wash hands with soap and water.

I. Exposures in the Laboratory

All suspected or known exposures must be appropriately documented on an Incident Report form and submitted to the Lab Manager promptly after the incident.

Biological Exposure

Remove any contaminated clothing or jewelry and wash the skin exposed to the agent with antibacterial soap.

Autoclave any contaminated clothing before disposal or further laundering. Decontaminate any surfaces by spraying the area with Lysol disinfectant or freshly prepared 10% bleach solution.

If you believe you may have been exposed to a biological agent, notify your instructor or the Lab Manager and seek immediate medical attention.

If you suspect you have a lab-acquired illness regardless of a recent exposure, seek medical attention.

Chemical Exposure

For most chemical exposures to the skin, remove any contaminated clothing and wash the chemical off with water for a minimum of 15 minutes. Safety showers should be utilized in the event of larger exposures.

J. Microbiology Culture List (BIOL 2420/2421)

Biosafety Level 1

Alcaligenes faecalis
Bacillus brevis
Bacillus coagulans
Bacillus licheniformis
Bacillus megaterium
Bacillus polymyxa
Bacillus sphaericus
Bacillus stearothermophilus
Bacillus subtilis
Citrobacter freundii
Clostridium sporogenes
Enterobacter aerogenes
Enterobacter cloacae
Escherichia coli
Flavobacterium capsulatum
Klebsiella oxytoca
Micrococcus luteus

Mycobacterium smegmatis
Providencia alcalifaciens
Pseudomonas fluorescens
Pseudomonas putida
Saccharomyces cerevisiae
Serratia marcescens
Staphylococcus epidermidis
Staphylococcus intermedius
Staphylococcus saprophyticus
Staphylococcus xylosum
Streptococcus bovis
Streptococcus mutans

Biosafety Level 2

Bacillus cereus
Edwardsiella tarda
Enterococcus faecalis
Klebsiella pneumonia
Morganella morganii
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Shigella boydii
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pyogenes
Streptococcus equi
Streptococcus equi subspecies
zooepidemicus

Resources

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition

<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

Guidelines for Biosafety in Teaching Laboratories

http://www.asm.org/images/asm_biosafety_guidelines-FINAL.pdf

Appendix to the Guidelines for Biosafety in Teaching Laboratories

[http://www.asm.org/images/Education/FINAL Biosafety Guidelines Appendix Only.pdf](http://www.asm.org/images/Education/FINAL_Biosafety_Guidelines_Appendix_Only.pdf)

Microbiological and Chemical Exposure Assessment

<http://www.epa.gov/nerlcwww/microbiology.html>

Laboratory Biosafety Manual - Third Edition

http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Culture Selection

<http://www.atcc.org/>

Laboratory Training Resources

Laboratory Biosafety Levels (FOCUS, Volume 5.1)

<http://cdc.train.org/DesktopModules/eLearning/CourseDetails/CourseDetailsForm.aspx?courseId=1030020>

Core Microbiology Skills

<http://cdc.train.org/DesktopModules/eLearning/CourseDetails/CourseDetailsForm.aspx?courseId=1035232>

Routine Microscopy

<http://cdc.train.org/DesktopModules/eLearning/CourseDetails/CourseDetailsForm.aspx?courseId=1046095>

Autoclave Safety <http://www.youtube.com/watch?v=T901F2W7wks>