The American Society of Microbiology (ASM) Education Board published Guidelines for Teaching Laboratories in 2012, and a revised document was released in 2019 (https://asm.org/guideline/asm-guidelines-for-biosafety-in-teaching-laborator). ASM put these biosafety guidelines in place in response to the ongoing release of Salmonella from teaching/clinical laboratories, as documented by the CDC in 2011, 2014, and 2017. The ASM guidelines include recommendations for working at Biosafety Level 1 and Biosafety Level 2. A major finding of the epidemiological investigation of the outbreak was deficiencies in biosafety awareness and proper training of staff and students. Rowan University has many teaching laboratories at introductory and advanced undergraduate levels, as well as graduate levels. Rowan University Environmental Health & Safety (EH&S) has compiled guidelines to ensure our teaching laboratories are safe for students and to prevent pathogen exposure to people and the environment.

This document contains biosafety requirements for teaching laboratories operating at Biosafety Level 1 (BSL1) and Biosafety Level 2 (BSL2). Not all teaching laboratories are equipped to safely operate at BSL2. Any and all use of BSL2 organisms must be pre-approved by the Rowan University Institutional Biosafety Committee (IBC) before any BSL2 work is performed in the teaching laboratory. Please contact the Biosafety Group in EH&S at ehs@rowan.edu with any questions or clarifications regarding assigned biosafety levels.

A word about subculturing "unknown" samples and teaching about differential and selective media:

The procedures needed to identify unknown microorganisms can be performed safely and with little to no risk to the students. Students are permitted to culture organisms from soil, water, food materials, and the air. Subculturing from the initial culture plate is permitted for the above samples, but IBC review and approval must be obtained if differential media used in the experiment could select for the growth of organisms listed at Risk Group 2 or higher. If the samples will be used to only count and understand the type of organisms in a particular environment, and no subculturing and isolation from environments such as water fountains, door handles, or other areas that could harbor pathogens, review and approval by the IBC must be obtained. Additionally, samples must never be cultured from the students themselves without prior approval from the Institutional Biosafety Committee, and possibly the Institutional Review Board, as there is a potential to grow organisms that require BSL2 or even Biosafety Level 3 (BSL3) containment.

It is recommended that testing of unknowns be performed from a mixture of known microorganisms (to the instructor) or from a culture whose contents are known to the instructor instead of from the environment.

For recommendations on surrogate microorganisms, please contact EH&S at ehs@rowan.edu.

BSL1 Requirements

Laboratory Facility Requirements:

- Non-porous floor, bench tops, chairs, and stools*
- Sink for handwashing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- RECOMMENDED: separate storage area for personal belongings (including cell phones)
- RECOMMENDED: access to a working and validated autoclave

*It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to include these requirements.

Stock Culture Requirements:

Stock cultures must be from authorized, commercial, or reputable sources. As indicated above, subculturing microbes isolated from the environment, clinical samples, or other unknown locations is discouraged as BSL2-classified microbes may be isolated. Subculturing from the environment must be reviewed and approved by the IBC.

Recommended Microbes and ATCC numbers:

Microorganism	BSL	ATCC number
Alcaligenes faecalis	1	8750
Aspergillus niger	1	16888
Bacillus globigii	1	
Bacillus stearothermophilus	1	7953
Bacillus subtilus	1	23857
Bacillus megaterium		
Citrobacter freundii	1	8090
Clostridium sporogenes	1	3584
Enterobacter aerogenes	1	13048
Enterobacter cloacae		
Enterococcus casseliflavus	1	700327
Enterococcus durans		
Escherichia coli B	1	11303
Escherichia coli K12	1	10798
Geobacillus stearothermophilus		
Halobacterium salinarum		
Klebsiella oxytoca	1	
Lactobacillus acidophilus		
Micrococcus luteus	1	4698
Neurospora crassa		
Penicillium chrysogenum	1	10106

Microorganism	BSL	ATCC number
Providencia alcalifaciens	1	
Pseudomonas fluorescens	1	
Rhanella aquatilus	1	
Rhizopus stolonifera	1	14037
Rhodococcus rhodocus	1	
Saccharomyces cerevisiae	1	9763
Sarcinia aurantiaca	1	
Serratia liquefacens	1	
Serratia marcescens Bizio	1	13880
Staphylococcus epidermidis	1	14990
Staphylococcus saprophyticus	1	15305

- Laboratory instructors must maintain documentation for all stock organisms, sources, and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis (at least annually) to ensure the source culture, minimize spontaneous mutations, and reduce contamination.
- Protocols that can be performed easily at BSL1: anaerobic growth, Gram stain, capsule stain, endospore stain, flagella stain, carbohydrate fermentation, casein hydrolase, catalase and oxidase test, bacterial enumeration, eosin methylene blue plate, gelatin hydrolysis, hanging drop, indole methyl red Vogues-Proskauer and Citrate (IMViC), Kirby-Bauer, Luria broth, litmus milk, 4-methyllumbelliferyl-b-D-glucuronide *Escherichia coli* broth medium (*E. coli* MUG*)*, MacConkey Agar, mannitol, nitrate reduction, spread, pour, quadrant streak plate, starch hydrolysis, transformation, plasmid DNA isolation, restriction endonuclease digestion, and gel electrophoresis.

Personal Protective Equipment (PPE) Requirements:

- Safety goggles or safety glasses (with side shields) must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash. If glasses are shared among students, they must be sanitized with an appropriate disinfectant after use.
- Long pants/skirts (ankle length), or other clothing to cover exposed skin must be worn.
- Closed-toe and closed-heel shoes that cover the top of the foot must be worn.
- Gloves must be worn when the student has fresh cuts or abrasions on the hands, when staining microbes, and when handling hazardous chemicals. Gloves are not required for standard microbiological work at BSL1 as long as proper hand hygiene is performed. Hands must be washed immediately after handling microbial cultures and at any time when accidental contact occurs with the skin. Hand cleansing must be completed with soap and water, and if none is available, with ethanol-based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.
- RECOMMENDED: Laboratory coats should be worn when handling cultures. In rooms where classes are working with risk group 2 agents, all people must wear laboratory coats at all times.

Laboratory Work Practices:

- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Lab benches must be disinfected upon entering the laboratory, when a spill occurs, and at the end of the laboratory sessions. Any materials that are spilled must be immediately cleaned up. Disinfectants used must be effective against microbes used in the laboratory. EH&S can be consulted for recommendations for disinfectants.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Do not touch your face, apply cosmetics (lip balm, etc.), adjust contact lenses, bite nails, or chew on pens/pencils in the laboratory.
- All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets, and other personal electronic devices is prohibited.
- Mouth pipetting is prohibited.
- All containers must be clearly labeled.
- The laboratory door must remain closed at all times when the lab is in session. The laboratory instructor must approve all people entering.
- Sharps usage must be minimized. Needles and scalpels are to be used according to institutional guidelines. All sharps (including coverslips, slides, and Pasteur pipets) must be disposed of in a sharps container.
- Test tube racks or other secondary containers must be used to move cultures in the laboratory.
- Stocks and other cultures must be stored in a leak-proof container when work is complete.
- Waste materials from the laboratory must be disposed of in accordance with University policy.
- Broken glass must be handled using a dustpan and broom or forceps/tongs, not by hand.
- All spills or injuries must be immediately reported to the laboratory instructor. Spills and injuries must then be reported to EH&S (ehs@rowan.edu).
- Teach, practice and enforce the proper wearing and use of personal protective equipment.
- Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation (students should not be asked to reveal if they are immune-compromised. A general announcement should be made that students with a reduced immune status should consult with the Wellness Center or their Primary Care Physician. A note from the Wellness Center or their Primary Care Physician is sufficient to excuse a student from laboratory work).
- RECOMMENDED: supply pens/pencils for students and keep them separate from personal items.
- RECOMMENDED: keep note-taking and discussion separate from work with laboratory materials.
- RECOMMENDED: use micro-incinerators rather than Bunsen burners.

Training Practices:

- Faculty, teaching assistance and all students must complete appropriate CITI training (https://research.rowan.edu/officeofresearch/compliance/cititraining/).
- Instructors and/or teaching assistants must review basic biosafety and microbiological practices with students on the first day of the lab. The requirements listed above must be included in this training session. The training session must be documented with a sign-in sheet maintained by the instructor. This can be performed using an online system.
- Students and instructors are required to handle microorganisms safely and in conjunction with the requirements outlined in this document.
- Inform students of safety precautions applicable to each exercise before the procedure is performed.

Documentation:

- Safety Data Sheets (SDSs) must be available in the laboratory for all chemicals.
- Require students to sign safety agreements indicating that they have been informed about all the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. The laboratory instructor must maintain students' signed agreements in the laboratory. Alternatively, this can be performed and maintained using an online platform.
- Prepare, maintain, and post caution signs on laboratory doors (complete with biohazard symbol).
- Instructors must provide students with a detailed list of microorganisms to be handled in the laboratory. This list can be included in the syllabus, laboratory manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.

Biosafety Level 2 Requirements

Biosafety Level 2 (BSL2) laboratories are suitable for working with microbes posing a moderate risk to the individual and a low community risk for infection. There are many microorganisms handled at BSL2 that can cause disease in humans via ingestion and inoculation. The guidelines for BSL2 laboratories build upon those for BSL1 facilities and typically include additional engineering controls to protect students, such as biological safety cabinets, centrifuge safety cups, and safety needle devices.

Laboratory Facility Requirements:

- Non-porous floor, bench tops, chairs, and stools*
- Sink for handwashing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- Separate storage area for personal belongings
- Working and validated autoclave (using biological spore indicators)
- Biohazard signage where cultures are used and stored (e.g., incubators), on the door to the laboratory and on containers used to transport cultures
- RECOMMENDED: Biological Safety Cabinet. Please see the requirements below. All biological safety cabinets must be certified by an approved vendor annually

*It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to include these requirements.

Stock Culture Requirements:

- Stocks must be authorized, commercial, or reputable sources. Do not subculture microbes isolated from the environment, clinical samples, or other unknown locations because they may be microbes that require BSL2 practices and facilities. Samples must never be obtained from clinical sites unless a full description of strain antibiotic susceptibility and resistance is provided. Prior approval from the IBC is required.
- Strains resistant to clinically relevant antibiotics may not be handled in teaching laboratories.
- Maintain documentation for all stock organisms, sources, and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis to ensure the source culture, minimize spontaneous mutations, and reduce contamination.
- Store cultures in a secure (locked) area
- Substitute surrogates for common BSL2 pathogens

Common Microbes and ordering information from ATCC:

Microorganism	BSL	ATCC Number
Proteus mirabilis	2	25933,7002
Proteus vulgaris	2	33420,8427,6380,49132
Salmonella enterica	2	700720
Staphylococcus aureus	2	12600

When choosing a test organism, many instructors want to choose organisms that are clinically relevant, i.e. pathogens. There are six microorganisms that are considered major threats, not because they cause the most devastating illnesses but because they comprise the majority of antibiotic-resistant infections observed in health care settings. These are referred to as ESKAPE pathogens and include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and species of Enterobacter (ESKAPE).*

ESKAPE pathogens	**>**	**Safe Relative**
Enterococcus faecium		*Enterococcus*
Staphylococcus aureus		*Staphylococcus*
Klebsiella pneumonia		*Escherichia coli*
Acinetobacter baumannii		*Acinetobacter baylyi*
Pseudomonas aeruginosa		*Pseudomonas putida*
Enterobacter species		*Enterobacter aerogenes*

Personal Protective Equipment Requirements:

- Safety goggles or safety glasses (with side shields) must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash. If glasses are shared among students, they must be sanitized with an appropriate disinfectant after use.
- Long pants/skirts (ankle length) or other clothing to cover exposed skin must be worn.
- Closed-toe and closed-heel shoes that cover the top of the foot must be worn.
- Laboratory coats must be worn. These can be disposable or made of cloth. Disposable coats may be reused but must be replaced when signs of damage or degradation are noticeable. Lab coats must be stored within the laboratory and must be assigned to individual students, not shared.
- Gloves must be worn when the student has fresh cuts or abrasions on the hands, when staining microbes, and when handling hazardous chemicals. Hands must be washed immediately after handling microbial cultures and at any time when accidental contact occurs with the skin. Hand cleansing must be completed with soap and water, and if none is available, with ethanol-based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.

Laboratory Work Practices:

- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Lab benches must be disinfected upon entering the laboratory, when a spill occurs, and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned up. Disinfectants used must be effective against microbes used in the laboratory. EH&S can be consulted for recommendations on disinfectants.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Do not touch your face, apply cosmetics (lip balm, etc.), adjust contact lenses, bite nails, or chew on pens/pencils in the laboratory.
- All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets, and other personal electronic devices is prohibited.
- Mouth pipetting is prohibited.
- All containers must be clearly labeled.

- The laboratory door must remain closed at all times when the lab is in session. The laboratory instructor must approve all people entering.
- Sharps usage must be minimized. Needles and scalpels are to be used according to institutional guidelines. All sharps must be disposed of in a sharps container (including coverslips, slides, and Pasteur pipets).
- Test tube racks or other secondary containers must be used to move cultures in the laboratory.
- Stocks and other cultures must be stored in a leak-proof container when work is complete.
- Students must be taught the proper techniques to minimize the production of aerosols. For example, when pipetting, place the tip on the side of the tube and allow liquid to run down the side of the tube. When flaming a loop to transfer culture, have a sterile agar placed as a "sizzle" plate so students do not touch a culture with a really hot loop.
- All procedures that generate aerosols, such as centrifuging, grinding, blending, shaking, mixing, sonicating, etc., must be performed inside a biological safety cabinet or using appropriate engineering controls (centrifuge safety cups). Biological safety cabinets must also be used when opening a container that can become depressurized and release aerosols of the stock culture.
- Waste materials from the laboratory must be disposed of in accordance with the University policy.
- Broken glass must be handled using a dustpan and broom or forceps/tongs, not by hand.
- All spills or injuries must be immediately reported to the laboratory instructor. Spills and injuries must then be reported to EH&S (ehs@rowan.edu).
- Teach, practice, and enforce the proper wearing and use of personal protective equipment.
- Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation (students should not be asked to reveal if they are immune-compromised. A general announcement should be made that students with a reduced immune status should consult with the Wellness Center or their Primary Care Physician. A note from the Wellness Center or their Primary Care Physician is sufficient to excuse a student from laboratory work).
- Supply pens/pencils for students and keep them separate from personal items.
- Keep note-taking and discussion separate from work with laboratory materials. If available, note-taking can be performed on a pull-out desk shelf, but it must be removed from the work area. If this is not available, a lecture must be performed before any materials are brought to the bench area.
- Use micro-incinerators rather than Bunsen burners. Bunsen burners are not permitted in biological safety cabinets. Micro-incinerators can also be used to heat fix bacterial smears on microscope slides and flaming the end of a test tube by passing these items over the entrance to the micro-incinerator.

Training Practices:

- Faculty, teaching assistants, and all students must complete appropriate CITI training (https://research.rowan.edu/officeofresearch/compliance/cititraining/).
- Instructors and/or teaching assistants must review basic biosafety and microbiological practices with students on the first day of the lab. The requirements listed above must be included in this training session. The training session must be documented with a sign-in

sheet maintained by the instructor. This can be performed using an online system.

- Students and instructors are required to handle microorganisms safely and in conjunction with the requirements outlined in this document.
- Inform students of safety precautions applicable to each exercise before the procedure is performed.
- Students are required to demonstrate proficiency in standard aseptic techniques at BSL1 practices and procedures before being allowed to work at BSL2.

Documentation:

- Safety Data Sheets (SDSs) must be available in the laboratory for all chemicals.
- Require students to sign safety agreements indicating that they have been informed about all the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. The laboratory instructor must maintain students' signed agreements in the laboratory. Alternatively, this can be performed and maintained using an online platform.
- Prepare, maintain, and post caution signs on laboratory doors (complete with biohazard symbol).
- Instructors must provide students with a detailed list of microorganisms to be handled in the laboratory. This list can be included in the syllabus, laboratory manual, or online at the course website.
- Register all work at BSL2 with the Institutional Biosafety Committee.
- Follow BSL2 practices and procedures as defined in the most current edition of the Biosafety in Microbiological and Biomedical Laboratories (https://www.cdc.gov/labs/bmbl/index.html)
- Emergency phone numbers and information must be posted in the laboratory.