BIOSC 1855: INTRODUCTION TO MICROBIOLOGY LAB
Department of Biological Sciences
University of Pittsburgh

Faculty & Staff
Tiffaney Czapski, Ph.D.
Office: L5 Clapp
Email: czapski@pitt.edu

Kimberly Payne, Ph.D.
Office: L8 Clapp
Email: kim.payne@pitt.edu

Office Hours
By appointment – send an email to your instructor specifying 2-3 times you can meet and check back for the reply/confirmation of the appointment.

Lab sections
<table>
<thead>
<tr>
<th>Mon/Wed: 11:00 - 12:50 pm (MW1100)</th>
<th>Tues/Thurs: 8:30 - 10:20 am (TR830)</th>
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<tr>
<td>1:30 - 3:20 pm (MW130)</td>
<td>11:00 - 12:50 pm (TR1100)</td>
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<td>4:00 - 5:50 pm (MW400)</td>
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Labs meet in G5 Clapp Hall.

Introduction
This is a survey course in laboratory microbiology, intended for students who would like to learn about the subject but do not plan to major in microbiology. Prerequisites are two semesters of introductory biology laboratory courses (at Pitt Biosci0050 and 0060) and co- (or pre-) requisite is a lecture course in general microbiology (Biosci1850). This 1 credit course has two laboratory meetings per week during the spring semester. The schedule of laboratory and lecture topics for each week is posted in the lab schedule section on CourseWeb.

Course Description
Students in this course will culture microorganisms from natural sources such as soil, vegetables, and their own skin, and learn various methods while studying the organisms they have cultured. The topics for the course include:

● Survey of types of bacteria
● Types of methods used to culture and study these microorganisms including aseptic technique; preparation of microbiological culture media; isolation of bacteria from natural sources and cultivation of various types of microbes; use of microscopes and basic staining techniques; identification of unknown bacterial isolates and some practical applications of microbiology such as identification of clinical isolates
● The exercises incorporate practice with (a) data analysis, (b) commonly used computational skills needed by microbiologists, and (c) safety practices for protecting the microbiologist, personnel on whom the laboratory depends (e.g. custodian and dishwasher), and the workspace from microbial contamination.

The curriculum includes a variety of standard methods that anyone having such a course listed on his or her transcript would be expected to know.

Course Goals
A student who successfully completes this course should be able to:

● Technical Goals:
Course Organization

- Perform standard lab techniques to culture, count, and characterize microbes.
- Use lab protocols typical in this field.
- Protect her/his own and others' safety while working with microbes and lab materials.
- **Knowledge Goals:**
  - Use basic technical language correctly.
  - List some of the commonly found microbes by name and give simple descriptions of them.
- **Reasoning Goals:**
  - Decide which of similar methods might be best to use in specific circumstances.
  - Develop skills in data analysis.

The Experiments: Most of the time, you will be conducting two or more experiments at once. This is standard practice in microbiology since inoculation occurs in the first period and the results can be interpreted in the subsequent periods after the culture has grown. The coursework is grouped into the following projects:
- Project 1: Basic techniques
- Project 2: Microbial physiology
- Project 3: Clinical microbiology

Course Material and CourseWeb

There are two required manuals for the course. Other materials needed for the class will be uploaded onto the CourseWeb.

Student Evaluation

Good laboratory work develops as a combination of many different skills. If you are better at the lab bench than at the examination desk, we want your grade to give you credit for that. Consequently, our evaluation of your coursework will proceed at several levels.
- **Preparation for class:** It is crucial that you are coming to the laboratory having read the day’s protocol(s). We expect you to read the protocols for a particular day's work BEFORE coming to class that day. If you don't do this, you will work unsafely and inefficiently and are likely to make major mistakes in your work.
- **Pre-Lab Assignments:** There will be multiple pre-lab assignments that will be assigned during the term and are listed on the lab schedule. These will be submitted electronically through CourseWeb and are due by the start of your class.
- **Examinations:** There will be written exams at the end of Projects 1 and 3. The Final Exam is TBD. It will be cumulative.
- **Practical examinations:** There will be hands-on laboratory practical exams for each Project. These will cover microscopy, aseptic technique, serial dilutions, physiological test protocols, spread plating, lab math, and preparation of Gram stained smears.
- **Assignments:** There will be scored assignments Projects 2 and 3 in which you may submit samples, experimental results, and analyses of experiments performed in class. The assignments will be submitted electronically.
- **Oral presentations**: There will be oral presentations that will take place throughout the semester. Groups of two or three students will present at a time. Preparation for the presentations will require individual as well as a team-work. Individual work will include research of an assigned microorganism or topic, and the team-work will entail coordinating the presentation, and drawing conclusions about the microorganisms and topics assigned to the group members.

- **Lab Notebook**: You will keep an ungraded lab notebook. This notebook will be your record of what you have done in the lab. It is not to be taken home except when explicit permission is given; rather all entries must be done during the class time. This policy follows ASM (American Society for Microbiology) regulations.

**Late Submissions**

Please be aware that, unless otherwise noted, late submissions of written and/or culture materials will be assessed a 10% penalty per date, i.e., a late but perfect submission 5 days after the deadline can earn only half the maximum points. Absolutely NO pre-lab assignments will be accepted late.

**Course Grade**

The grade will be approximately as follows (and adjusted with notice as needed):

- Pre-Labs ........................................... 15%
- Oral Presentation .................................. 10%
- Assignments ....................................... 20%
- Practical Exams .................................... 20%
- Project exams, Final Exam ....................... 30%
- Competence points ................................ 5%

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<th>Grade</th>
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<td>A+</td>
<td>98-100</td>
<td>C+</td>
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<td>B+</td>
<td>88-89</td>
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<td>B</td>
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Historically, final grades fall on the following grading scale. HOWEVER, final grades are NOT constricted to these requirements. Your final grade is at your instructor's discretion.

- Grades are a reflection of the quality of your performance in this class. Grades are not a reward for effort or perfect attendance.
- As the semester progresses, we expect you to improve in your bench skills, and in your competency with reading and following a protocol. We will give you feedback, but you should develop independence and self-analysis.
- You are responsible for submitting complete assignments and pre/post lab assignments that follow the guidelines and instructions.
- You are responsible for preparing for the practical and written exams.

**Final Exam Conflict**

Visit the Course Policies in CourseWeb for the University’s guidelines and forms. Please speak with your instructor immediately since there are university deadlines for final exam conflict resolution.

**Contesting a Grade**

If you wish to contest a grade for any material that is evaluated in this course, you may do so by submitting your request in writing and explaining why you think the
grading was in error. You must include a detailed justification for the correctness of your answer, including references to the text used in the course (text, page, paragraph), unless the re-grade is due to an addition error. This request must be submitted to the instructor within one week from the return of the materials. Please be aware that the entire document may/will be re-graded.

Late Add Policy

Since experimentation and other class activities start on the first day of class, if you add the class during the Add/Drop period, you will have missed important course work. Be aware that you are responsible for catching up and completing any missed work. The deadlines for any project-associated work and submissions will not be extended. There are no opportunities for make-up lab time or missed assessments. Your instructor will be as accommodating as possible to meet with you outside of your normal class time; this arrangement must be initiated by you.

Absences

- If you aren’t certain whether your reason for an absence will meet our guidelines, please ask. We try to be as accommodating as we can while still treating everyone fairly. Any need for absence from a practical/exam or request for extra time on an assignment must be documented.
- If you miss any classes or work due to a mental health issue, we do ask for documentation so we can accommodate you as best as we can for each instance. A great resource for students is the University Counseling Center and the services provided are free of charge. It offers a variety of mental health services to students utilizing a short-term, time limited approach, including assessment, counseling and psychotherapy services (individual, group, and couples), and psychiatric services. Their website is: http://www.studentaffairs.pitt.edu/cchome
- If you miss a practical/exam and are permitted to take a makeup, the makeup may not be of the same kind as the exam taken by the class. For example, it could be composed of essay questions instead of short answers or it might be an oral exam.
- If you will be unable to attend any day that material is scheduled, we may or may not be able to arrange for you to make up the work, depending on what experiments are involved. Some class materials are unstable and complicated to prepare and we may not be able to make them available at additional times.
- Absences will not excuse you from not being responsible for the material missed for assignments, practical exams, or other examinations.
- Since the background material is usually presented at the start of the lab session, missing the start of a class counts as a minimum of one half of the period missed.
  - If you arrive late, you will be allowed to participate in the day’s class only at the discretion of the instructor.
  - If your tardiness results in a safety concern and/or an undue instructional burden, you will not be allowed to attend class on that day. In such a case, it will be your responsibility to arrange for a time to make up the work.
- Office hours are intended for meetings with instructors for help and discussion on experiments; they are not to be used for making up missed work!
- If you miss a class because of an emergency, please let us know as soon as you can. Please call (or ask someone else to call), or send an email to your instructor. The preferable contact method is by email.
- It is unlikely that a single unexcused absence will have much effect on your grade. Keep in mind however, that an unexcused absence is unacceptable and impossible to overlook. Any incomplete lab exercise will affect your grade.
- Missing more than 10% of the laboratory periods will result in a failing grade (‘F’) even if the absences are excused and no matter how well you do on the examinations and assignments.
Competency

Infractions of classroom behavior, class etiquette (see section below), and safety procedures will be documented and, at the instructor’s discretion, points will be deducted. Additionally, if your bench work competency is deemed to be unsatisfactory, you will not be allowed to proceed to the clinical unit (Project 3) with the use of wild bacterial isolates, but will instead be assigned laboratory strains. Any grade consequences are outlined in the "Consequences" section of the Syllabus.

Class Etiquette Includes:

- Loop incinerators & inoculating loops:
  - Turn on the loop incinerator at the start of class.
  - Turn off the loop incinerator at the end of class.
  - 5-10 seconds of sterilization time ONLY!
  - Absolutely no “parking” of inoculating loops or needles in the loop incinerators.
  - If left in the loop incinerator for >30 seconds, they will melt, be destroyed, and damage the loop incinerator.

- Test tubes & racks:
  - Not all of the test tube caps are tight – do not carry the test tubes by the caps.
  - If carrying more than two test tubes, use a rack.
  - When finished, consolidate the test tubes to be discarded in the racks on the waste cart and return the racks to the storage location.

- Staining trays & racks:
  - Stainless steel trays and racks must be rinsed with water (or ethanol if the stains do not rinse off with the water), dried, and returned to the storage location when you are finished staining.

Consequences

As in all aspects of life, there are consequences for your actions. When you are employed in the healthcare and/or science field the consequences for inappropriate actions can be severe. A patient may get sick or sicker; you may get sick; a colleague may get injured; you may get fired.

The laboratory techniques and behaviors that you learn and practice in this class will be carried forward into your professional life. You will be given instruction and guidelines on many microbiological and clinical techniques and procedures. You are expected to pay careful attention to the instruction, and to practice the techniques and procedures. The instruction and practice are designed to keep you, your classmates, your instructors, and your future patients and colleagues safe.

You will start the semester with 10 Competence Points, worth 5% of your final grade. If you are attentive and careful, you will keep all of your competence points. Competence points will be subtracted for violations of any safety procedure. This includes, but is not limited to, improper aseptic technique and incorrect waste disposal (see the separate document dealing with proper waste disposal). You will lose competence points if you are inattentive to protocol details; this results in contaminated strains, useless experimental results, waste of reagents and other experimental materials. You will also lose competence points if you do not learn and practice proper care and use of the laboratory equipment, including, but not limited to, the microscopes, micropipettes, and common areas. You will lose competence points for violations of classroom etiquette and behavior such as improper care and use of classroom supplies, media and solutions, as well as glass and plastic ware.

Each student will have three “warnings” before they begin to lose competence points. These are visible in the Grade Center in CW. Repeated offenses will incur higher point losses. The competence points are tied to your instructor’s assessment of your
Plagiarism is a serious offense that will not be tolerated in this class or at the University. If plagiarism is seen, points will be deducted immediately and cannot be earned back. Any plagiarism will result in partial or full loss of points for the document where the plagiarism occurred. If the issue continues, the offenses will be brought up to the Chair of the Biological Sciences, Jeffrey Lawrence, the Director of Undergraduate Programs, Valerie Oke, and the Dean of Students.

For an explanation of what plagiarism is, please visit the website: http://plagiarism.org. For anything you look up for any class work, cite your references and use your own words! This also includes the class textbook and materials posted on CourseWeb.

The instructor will not keep any course materials (exams, notebooks, assignments) beyond four weeks after the close of the term. If you wish to have any course materials returned to you, please contact the instructor before the time period ends.

In keeping with the University of Pittsburgh Student Code of Conduct, all students are expected to behave as respectful and civil members of the university community. All instructors and students will act in a considerate manner in order to create and maintain a classroom atmosphere that is conducive to learning. In addition to being unacceptable on the grounds of common decency, disruptive and disrespectful behavior contributes to unsafe working conditions.

Disruptive and disrespectful behavior will not be tolerated. Examples of disruptive behavior include, but are not limited to, repeated tardiness, texting in class, speaking or acting in any sexually, racially, or ethnically harassing manner, cheating, misuse and abuse of laboratory equipment and material, disregarding any safety guidelines. Disruptive students will be referred to the Office of Student Conduct for mediation, discipline, or both.

Each student is issued a University e-mail address (username@pitt.edu) upon admittance. This e-mail address may be used by the University for official communication with students. Students are expected to read e-mail sent to this account on a regular basis. Failure to read and react to University communications in a timely manner does not absolve the student from knowing and complying with the content of the communications. The University provides an e-mail forwarding service that allows students to read their e-mail via other service providers (e.g., Hotmail, AOL, Yahoo). Students that choose to forward their e-mail from their pitt.edu address to another address do so at their own risk. If e-mail is lost as a result of forwarding, it does not absolve the student from responding to official communications sent to their University e-mail address.

To forward e-mail sent to your University account, go to http://accounts.pitt.edu, log into your account, click on Edit Forwarding Addresses, and follow the instructions on the page. Be sure to log out of your account when you have finished. (For the full E-mail Communication Policy, go to www.bc.pitt.edu/policies/policy/09/09-10-01.html.)

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and the Office of Disability Services.
Resources and Services, 140 William Pitt Union, 412-648-7890/412-624-3346 (Fax), as early as possible in the term. Disability Resources and Services will verify your disability and determine reasonable accommodations for this course. For more information, visit www.studentaffairs.pitt.edu/drsabout.

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Accessibility
Blackboard is ADA Compliant and has fully implemented the final accessibility standards for electronic and information technology covered by Section 508 of the Rehabilitation Act Amendments of 1998. Please note that, due to the flexibility provided in this product, it is possible for some material to inadvertently fall outside of these guidelines.

Gender Inclusive Language
Language is gender-inclusive and non-sexist when we use words that affirm and respect how people describe, express, and experience their gender. Just as sexist language excludes women’s experiences, non-gender-inclusive language excludes the experiences of individuals whose identities may not fit the gender binary, and/or who may not identify with the sex they were assigned at birth. Identities including trans, intersex, and genderqueer reflect personal descriptions, expressions, and experiences. Gender-inclusive/non-sexist language acknowledges people of any gender (for example, first year student versus freshman, chair versus chairman, humankind versus mankind, etc.). It also affirms non-binary gender identifications, and recognizes the difference between biological sex and gender expression. Students, faculty, and staff may share their preferred pronouns and names, and these gender identities and gender expressions should be honored.

Recording Statement
To ensure the free and open discussion of ideas, students may not record classroom lectures, discussion and/or activities without the advance written permission of the instructor, and any such recording properly approved in advance can be used solely for the student’s own private use.

Waste Disposal Guidelines

Soft Waste
● Dispose of ALL paper and soft waste, such as KimWipes and Optik Wipes, in the plastic bins for soft waste located on the bench.
● All used, contaminated toothpicks should be discarded in the designated bench top biohazard container. Any unused toothpicks in containers should be placed on the waste cart for re-autoclaving.
● Any biological sample, saliva or urine, MUST be discarded in the biohazard bins ONLY by the person who supplied the sample.

Glass Waste
● All broken glass is to be discarded in the broken glass boxes. There are large boxes located near each door.
● All used glass slides, including staining slides with bacteria, should be discarded in the broken glass boxes. They DO NOT go into the biohazard container.
● Broken test tubes should be discarded in the broken glass box. All other test tubes used in class are placed and consolidated in a rack at the designated location. Return empty racks to the proper cabinet.
● NEVER write on test tube caps (write on glass test tube), and do NOT discard in
biohazard or soft waste containers.

- Glass serological pipettes, not the disposable pipettes, should be placed tip down in the pipette discard containers located at every bench. Put the canister, once opened, on the waste cart to be re-autoclaved.

**Plastic Waste**
- Any used but uncontaminated plastic material, i.e.: Eppendorf tubes, pipette tips, PCR tubes, should be placed in the soft waste bins.
- Any contaminated plastic waste should be discarded in the biohazard bags at your bench or in the large biohazard bins.
- Place any plastic containers that are empty or need to be re-autoclaved on the waste cart. Do not throw the containers in the garbage or biohazard bins. Do not replace the containers on the supplies bench.

**Media**
- Any used or unusable plates should be discarded in the large biohazard bin.
  - If a plate was not used, e.g. you grabbed one too many plates, return the plate to the supplies bench. It can be used by another student. Do not just discard and be wasteful.
- Any used or unusable media-filled test tubes should be consolidated in a rack on the waste cart. **Glass should never be discarded in the biohazard bins.**
- Any milk dilution bottle containing media should be placed on the waste cart even if there is leftover media. It must be re-autoclaved before the next use.
- Do not replace the used milk dilution bottle back on the sterile supplies bench.

**Chemicals**
- All reagents must be discarded in the labeled receptacle. DO NOT put any chemicals/reagents in the biohazard or soft waste bins unless specified.
  - Gram stain reagents collected in the stain trays should be discarded into the staining reagent discard container located near the sink. After the reagents are discarded, rinse the tray with water that should be flushed down the sink. Completely dry the tray before returning it to the common drawer.
  - Certain tests require the use of syringes to add chemicals. DO NOT move or discard the syringe. Do not cap or re-cap the needle. Please leave this for the instructor.

**Penalties**
- Any failure to follow the waste disposal guidelines listed above will result in one warning given by the instructor. A failure to comply after the initial warning may result the deduction of Competence Points.
- If improper disposal continues after a grade deduction, the instructor has the ability to judge the student as incompetent in a laboratory setting. Incompetency will result in the student being unable to perform advanced tests. At the discretion of the instructor, this WILL affect the course grade.
- Any grievous offense determined by the instructor, e.g. improper disposal causes a person bodily harm, may result in failure of the class. The incident can and will be documented and brought to the attention of the Chair of Biological Sciences and the Dean of Students.
- Safety in the laboratory is the instructors’ and the University’s top concern. If you are unsure of the proper disposal of material, ASK!!
- Following strict guidelines is not only essential for teaching labs, but will be critical for your future professional career as researchers or health professionals.
Microscope Care and Use

- **When you take your microscope out of the cabinet:**
  - Check to make sure it had been properly stored.
  - Clean the oculars and all the objective lenses with Optik-Wipes.

- **When using your microscope:**
  - Use the light knob (rheostat) to turn up the light to about ¾ of maximum.
  - Use the diaphragm lever for light adjustment when moving from lower to higher magnification. You may need to open the diaphragm to allow more light through as you move to higher magnifications.
  - Since our compound microscopes are parfocal, your specimen should stay in focus, or very close to in focus, when moving between magnifications; therefore, you should always start examining your sample using the lowest lens where you have the largest depth of field.
  - When moving to a higher magnification, do not move the stage up or down, just swing the next higher lens into place. You may have to adjust the fine focus, but should not move the coarse focus.
  - When using the 100X (oil immersion) lens, do not move the stage up or down, move all lenses out of the way and place a drop of immersion oil just above the stage aperture (you should be able to see light coming through the aperture). Watch closely while you swing the 100X lens into the oil in order to ensure that you do not smash the lens into the slide. Again, you may need to adjust the focus, but should not have to move the coarse focus.
  - Since the depth of field is very shallow with the higher magnification lens, it is important that you initially focus at the lower power or you may never find your sample at the higher power.
  - Remember, the 100X lens is meant to be used with oil; you will not be able to see your sample clearly without it.
  - **Do not remove the glass slide without first lowering the stage.** You may damage the lens if the glass slide scrapes across it.

- **When you put away your microscope, please do all of the following:**
  - Remove any slides or other specimens from the stage.
  - Set the objective lens to the lowest power on the scope.
  - Clean the stage, oculars and all the objective lenses with Optik-Wipes.
  - Move the stage all the way up.
  - Put in the cabinet with the handle facing out.

- **Penalties will be imposed for improper use and storage of microscopes.**

To avoid injury to yourself and fellow students you are required to read, understand, sign, and abide by this agreement. Failure to comply with these rules while performing laboratory experiments may result in suspension or expulsion from this laboratory course.

Microbiology Lab Safety Instructions

1. NEVER EAT, DRINK, OR SMOKE IN THE LABORATORY.
2. If you have a medical problem or condition that may affect your performance or safety in the laboratory you must discuss it in private with your Laboratory Instructor. This information will be held in strict confidence.
3. You must wear closed-toed, full shoes in the laboratory. BARE FEET OR ANY
TYPE OF SANDALS CANNOT BE WORN INTO THE LABORATORY. Failure to comply with this rule will result in your dismissal from lab for the day.

4. YOU MAY NOT WEAR SHORTS THAT COVER LESS THAN HALF OF THE THIGH WHILE SITTING, CUT-OFF TEE SHIRTS, MIDRIFF TOPS OR HALTERS IN THE LABORATORY. If you are dressed inappropriately for lab, you will be dismissed from lab for the day.

5. You MAY NOT PIPE any solution BY MOUTH.

6. NOTIFY YOUR INSTRUCTOR IMMEDIATELY of any accident or spill.

7. Note the location safety showers so that you can use them if needed.

8. Eye injuries, whether chemical or mechanical, must always be considered to be serious. The best procedure, in case of chemical injury to the eye is immediate, prolonged, continuous flushing with water (15-20 min) at an eye fountain, which should be located on the first day. Eyes must be forced open to be washed well.

9. If you get any chemicals or cultures on your hands WASH YOUR HANDS IMMEDIATELY. Rapid and immediate treatment is essential. Clothing soaked with strong acid or alkali MUST immediately be removed. THIS IS NO TIME FOR MODESTY. The safety showers are mainly intended to be used in cases where corrosive chemicals are spilled or splashed over a large body area.

10. Give cracked or chipped glassware to your Instructor. Broken glassware MUST BE PLACED IN THE “BROKEN GLASS” BOX ONLY.

11. Never force glass rods, pipets, or tubing into rubber stoppers. USE PROPER TECHNIQUE AND CARE WHEN INSERTING A PIPET INTO A PIPET BULB OR ELECTRIC PIPETTOR.

12. For emergency treatment of any accident you must go to Presbyterian University Hospital. Transportation will be provided if needed — speak with your Instructor. You and your Lab Instructor together must file an Accident Report within 24 hours. Non-emergencies will be handled at Student Health.

13. Exercise great care in noting the odor of fumes, and AVOID BREATHING FUMES OF ANY KIND. Use fume hoods when necessary.

14. Long hair must be confined securely to minimize hazards.

15. DO NOT RUN or “horse around” in the laboratory. Do not engage in any activities or behavior that might confuse, startle, or distract another student.

16. DO NOT PUT ANY CHEMICAL, SOLID OR LIQUID, BACK INTO THE STOCK BOTTLES from which they were obtained. The excess chemical may now be contaminated. Ask your Instructor what to do with the excess chemicals.

17. NEVER REMOVE chemicals from the laboratory.

18. IF A FIRE ALARM SOUNDS while you are working in the laboratory, turn off any Bunsen burners, loop incinerators or other electrical appliances and leave the building by the nearest exit.

19. WASH YOUR HANDS BEFORE YOU LEAVE LAB. It is a good idea to wash your hands whenever they have been in contact with any chemical or culture, not just at the end of the lab period. When you leave for the day, use the BacDown antibacterial soap provided.

20. Always clean your work area at the beginning and end of each lab period. Use the disinfectants provided.

21. An Instructor must always be present while students are working in the lab. YOU ARE NOT PERMITTED TO DO UNAUTHORIZED EXPERIMENTS.

22. Except in very unusual circumstances, all medical claims are the responsibility of the student. INSURANCE COVERAGE by either a student plan or family plan is strongly recommended.