

# **BIMM 121 Laboratory in Microbiology**

## **Fall 2012**

4070 C York Hall  
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858-822-2032

**Office hours:** Mondays 11:30am-12:45 pm. Location: 4070C York Hall

**Lecture:** Tuesday/Thursday 8:00 – 9:20 in PCYNH 122 (Pepper Canyon Hall, by the Gilman Parking Structure)

**Labs:** York 2310 and 2332  
Tuesday/Thursday: 9:30 am – 1:30 pm  
Wednesday/Friday: 10:00 am – 2:00 pm

### **Course Structure:**

This course will introduce you to the fundamentals of microbiology and allow you to explore the many ways in which microbes affect and are used in our lives. We begin the course with a foundation in basic techniques such as sterile techniques, microscopy, methods of quantitating microbes, and preparing and examining stained slides. The remaining duration of the course will comprise four main units: a comprehensive look at bacterial physiology, understanding the complex microbial community of soil, metagenomics as a tool in exploring complex communities, and the use of microbes in various aspects of our lives. Each of these units comprises several multi-day experiments and there will be considerable overlap in the execution, methodology, and analysis of data from each of these units. Throughout the course, you will also receive training in accurate data entry and analysis, scientific reasoning, and in clear and concise scientific writing.

### **Equipment:**

For this lab you will need to purchase:

- A lab notebook (bound notebook, regular or spiral bound). Carbon notebook not necessary. Loose leaf binders not allowed.
- A lab coat
- Eye protection (you may wear either safety glasses or goggles, but standard prescription eye glasses are not sufficient).
- A Sharpie permanent marker pen, preferably fine point (not extra fine or regular)

### **Attendance and Absences:**

1. Your attendance is required at EVERY lab and through the entire lab period, until all the experimental work for the day is completed.
2. Absences will NOT be treated lightly. The labs are set up for groups of two or more and your absence will place an unnecessary burden on your partner. There are no make up labs and you will not be allowed in the lab on non-lab days or in the other Micro lab sections, although you may be asked to make up the work from the day you missed.
3. Documentation will be required for all unavoidable absences.
4. If you are likely to have interviews for graduate school, etc., please schedule them on non-lab days.
5. All absences without prior notification/permission and the appropriate paperwork will be considered unauthorized.
6. **50-point penalty** for the first unauthorized, unexplained absence from the lab. If there is a second such absence, you will be asked to drop the course or will be given an F.
7. If you are ill on a lab day or have an emergency, e-mail or call (instructor or lab partner) before the start of the lab. If you are ill enough to miss lab you must go to the student health center and provide documentation of your illness.

### **Homework and Lab report Deadlines and Submission:**

1. A hard copy of each homework/lab report is due in the first 5 minutes of the lab period of the day on which your report is due. Reports/homework turned in more than 10 minutes after the start of class will be considered late. Penalty for late reports will be 10% for each day late.
2. In addition to the hard copy of the report, you are required to submit an electronic copy to Turnitin.com. A link to the e-submission website will be provided on Ted. Failure to submit on Turnitin.com will result in 0 (zero points) recorded for that report. Check the deadline of the Turnitin.com submission and make sure you adhere to it. Students agree that by taking this course all required papers would be subject to review for textual similarity by Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin.com service is subject to the terms of use agreement posted on the Turnitin.com site. Some homework assignments also require Turnitin.com assignments.
3. Additional points may be taken for late electronic submissions.

### **Regrade Requests:**

All regrade requests should be submitted in writing within one week of receiving the graded material.

## Grading Scheme

Quiz/Report/Midterm	Points	% of total
Classroom evaluation	40 points	6.2
• Notebook checks	20 points	
• TA eval	10 points	
• Lecture participation	10 points	
Quizzes and practicum	100/110 points	15.4/17
8 reg + 1 math + 1-2 practicum?		
80 (11%) + 10 (2%) + 10 or 20 (1.5 - 3/%)		
Homework	150/160 points	23/25
Lab Report (1)	100 points	15.4
3 Midterms	<u>250 points (70 + 95 + 85?)</u>	38.5
<b>Total</b>	<b>650 points</b>	

### Homework

HW1	Pre- lab survey	10 points
HW2	Baseline data analysis	10 points
HW3	Library tutorial	20 points
HW4	Water contamination analysis	40 points
HW5	Dilution problems	10 points
HW6	Unknown analysis + research	40 - 50 points
HW7	Growth curve	10 points
HW8	Post-lab survey	<u>10 points</u>
	<b>Total</b>	<b>150 - 160 points</b>

## Most Likely Grade Distribution

A = 90% - 100%

B = 80% - 89.9%

C = 70% - 79.9%

D = 60% - 69.9%

F = below 60%

### Notebook:

Spiral bound or composition notebook is OK. All notebooks should have a table of contents (handwritten OK) so on the first lab day leave several blank pages at the beginning of your notebook. Number your pages. Entries should be made in chronological order and EVERY day. Each day's entries on each experiment

should begin with a brief (1 – 2 sentences) summary of work done on the same experiment the previous day.

### **How to use your notebook**

Table of contents – update everyday – leave at least 4-5 pages for updating

Start a new page each day for each new experiment:

Purpose of experiment

Procedure

Outline or page from which protocol was taken

Note any changes

Note who did which part of the procedure – who inoculated controls, etc

Note which organisms you used – name and species of the controls, etc

Errors

Observations

Write – in detail

Draw – enlarged, labeled, and including as much detail as possible

Questions and connections

Conclusion or summary

Answer any questions in the manual or that were raised in class.

Number your pages

You may leave space to complete an experiment. When the experiment is complete and all observations have been made, cross off any blank pages or parts of pages following the written portion.

### **Lab Performance and Participation**

In addition to quizzes, midterms, lab reports and homework assignments, student evaluations will be based on the following criteria:

1. Lab techniques will be evaluated in class
2. Lab workshop participation

Subjective student evaluations will be based on the following criteria:

3. Pre-lab preparation
4. Careful management of lab procedures (e.g., sterile technique, proper waste disposal, experimental procedures, etc.)
5. Ability to adapt to unforeseen procedural changes
6. Caliber of thinking before asking questions

7. Scientific approach (e.g., proper use of notebooks, controls, experimental design)
8. Accuracy
9. Independence
10. Safety consciousness
11. General neatness in lab

Please note: **You will be expected to get into the habit of methodical, well-planned and organized work by the mid-term. This will help you with the experiments in the second half of the course.**

## **Course Website**

This course is on Ted (<https://ted.ucsd.edu>) and should automatically appear on your Ted account as soon as you register for the class. We will use Ted to post information on experiments, exams, schedules, readings and practice material, experimental data, report guidelines, etc. We strongly encourage you to use the Discussion board to post questions or answers to questions and to use it as a forum for exploring the material. The TAs and I will routinely check this website and answer any questions but feel free to respond as well. This website will also be used to post any announcements that pertain to the entire class. Please check the site regularly and update yourself on the information provided.

## **University Policy on Integrity of Scholarship**

The principle of honesty must be upheld if the integrity of scholarship is to be maintained by an academic community. The University expects that both faculty and students will honor this principle and in so doing protect the validity of University grading. This means that all academic work will be done by the student to whom it is assigned, without unauthorized aid of any kind. Instructors, for their part, will exercise care in planning and supervising academic work, so that honest effort will be encouraged.

### **Student Responsibility:**

Students are expected to complete the course in compliance with the instructor's standards. No student shall engage in any activity that involves attempting to receive a grade by means other than honest effort; for example:

- No student shall knowingly procure, provide, or accept any unauthorized material that contains questions or answers to any examination or assignment to be given at a subsequent time.
- No student shall complete, in part or in total, any examination, or assignment for another person.

- No student shall knowingly allow any examination or assignment to be completed, in part or in total, for himself or herself by another person.
- No student shall plagiarize or copy the work of another person and submit it as his or her own work.
- **If any work is plagiarized from that of another student, both students will be reported to the Office of Academic Integrity, even if one of the students has graduated already. Remember that most graduate schools check the undergraduate records for any indications of dishonesty before awarding a degree.**
- No student shall alter graded class assignments or examinations and then resubmit them for regrading.
- No student shall submit substantially the same material in more than one course without prior authorization.

<b>Week/Lab</b>	<b>Date</b>	<b>Experiment</b>	<b>Reports, Midterms, Reminders</b>
Week 0 Lab 1	Thurs/Fri Sept 27/28	Registration, attendance, safety video, responsibility agreements, introductory remarks, Safety lecture  <b>Sterile technique.</b> Microbes in the environment Why wash your hands? <b>Use of pipettors:</b> Demo and exercise <b>Plant pathogen interaction:</b> Inoculate <i>Kalanchoe</i> plant with <i>Agrobacterium</i>	<b>HW 1 due Fri 28<sup>th</sup> midnight: pre-workshop library survey</b>
Week 1 Lab 2	Tues/Wed Oct 2/3	<b>Sterile technique.</b> Microbes in the environment: Observe results <i>E.coli</i> and toilet paper experiment: Observe results Aseptic technique: streak and spread plates Demo Lab exercise using a mixed bacterial culture  <b>Microscopy:</b> Learning to focus the light microscope	<b>Quiz 1</b>

		<p>Demo</p> <p>Lab exercise using prepared (commercial) slides</p> <p>Cleaning your microscope – demo and completion</p>	
<p>Week 1</p> <p>Lab 3</p>	<p>Thurs/Fri</p> <p>Oct 4/5</p>	<p><b>Microscopy:</b></p> <p>Calibrating your microscope: Demo and complete</p> <p>Making a wet mount and Phase Contrast Microscopy: Wet mounts and phase contrast: - view, identify, and measure (all with Hay Infusion)</p> <p><b>Understanding dilutions:</b></p> <p>Understanding dilutions- theory</p> <p><b>Measuring microbial growth: Yeast</b></p> <p>Direct counts using a hemocytometer</p> <p>Using a spectrophotometer</p> <p>Counting viable cells using plating</p>	<p><b>HW2 due – table/figure from toilet paper data</b></p>
<p>Week 2</p> <p>Lab 4</p>	<p>Tues/Wed</p> <p>Oct 9/10</p>	<p><b>Microscopy:</b></p> <p>Continue/complete all wet mounts (all other bacterial and yeast)</p> <p><b>Microscopy: Staining</b></p> <p>Smear preparation and simple staining</p> <p>Gram stain: control organisms only</p> <p><b>Characterizing the Unknown Organisms:</b></p> <p><b>Introduction:</b> Receive unknown organisms: make a wet mount, streak plate and set up broth culture with organisms</p>	<p><b>Quiz 2</b></p>
<p>Week 2</p> <p>Lab 5</p>	<p>Thurs/Fri</p> <p>Oct 11/12</p>	<p><b>Characterization of the Unknown Organisms</b></p> <p>Streak stock TSS slant, do wet mounts from both temperatures</p> <p><b>Microscopy: Staining</b></p> <p>Complete staining of designated Gram positive and Gram negative controls</p> <p><b>Characterization of the Unknown Organisms</b></p> <p>Gram stain</p> <p>MacConkey – inoculate along with known</p>	<p><b>HW 3 due – online library tutorial</b></p> <p><b>Read coliform chapter</b></p>

		G+ and G- organisms Sticky test along with known G+ and G- organisms Endospore test – inoculate NSM	
Week 3 Lab 6	Tues/Wed Oct 16/17	<b>Data Analysis Workshop:</b>	<b>Quiz 3 (10)</b>
Week 3 Lab 7	Thurs/Fri Oct 18/19	<b>Inoculation of control organisms (to create fresh stocks):</b> <i>Enterobacter aerogenes</i> <i>Escherichia coli</i> <i>Proteus vulgaris</i> Inoculate 1 TSS or TSA of each per aisle. These slants will be used for the controls for the urease test on Lab 8 <b>Characterization of the Unknown Organisms</b> NSM – Complete <b>Macronutrient use – how organisms get energy to survive:</b> Introduction: Hydrolysis and use of large extracellular materials <b>Polysaccharides:</b> Starch plates - inoculate <b>Proteins:</b> Skim milk plates and gelatin deeps - inoculate <b>Lipids:</b> Rhodamine plates - inoculate	<b>MT 1 in lecture? Lab? Check with instructor</b>
Week 4 Lab 8	Tues/Wed Oct 23/24	<b>Fundamentals of library research: 90 minute hands on workshop</b> <b>Characterization of the Unknown Organisms:</b> <b>Macronutrient use – how organisms get energy to survive</b> Polysaccharides: Starch plates - complete Proteins: Skim milk plates and gelatin deeps – complete Lipids: Rhodamine plates – complete <b>Special metabolic functions: Unknown organisms</b> Indole production from tryptophan, catabolite repression – inoculate	<b>Quiz 4</b> <b>Workshop in York 3010 confirmed</b> <b>Lab in usual location</b> <b>Oct 23<sup>rd</sup> 9:30 – 11:30</b> <b>Oct 23<sup>rd</sup> 5:00 – 6:30</b> <b>Oct 24<sup>th</sup> 10:00 – 11:30</b>



		Urease test – inoculate Differential utilization of citrate by enterics - inoculate	
Week 4 Lab 9	Thurs/Fri Oct 25/26	<b>Characterization of the Unknown Organisms:</b> <b>How energy is produced – aerobic vs. anaerobic breakdown of organic compounds</b> Acid and gas production from sugar fermentation – inoculate Methyl-Red and Voges-Proskauer – inoculate <b>Special metabolic functions: Unknown organisms only</b> <ul style="list-style-type: none"> <li>• Indole production from tryptophan, catabolite repression –</li> <li>• complete</li> <li>• Urease test – complete</li> <li>• Differential utilization of citrate by enterics – complete</li> </ul> <b>Inoculation of control organisms (to create fresh stocks):</b> <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> Inoculate 1 TSS slant of each per aisle. These slants will be used for the controls for the nitrate test on Lab 10 <b>Motility</b> – inoculate plate and deep with test organism	<b>HW4 due – post workshop longer table homework</b>
Week 5 Lab 10	Tues/Wed Oct 30/31	<b>Characterization of the Unknown Organisms:</b> <b>Inoculation of control organisms (to create fresh stocks):</b> <i>Escherichia coli</i> <i>Pseudomonas fluorescens</i> <i>Enterococcus faecalis</i> <i>Staphylococcus epidermidis</i> Inoculate 1 TSS or TSA of each per aisle. These slants will be used for the controls for the Cyto C and catalase tests on Lab 11	<b>Quiz 5</b> <b>Additional lecture/review time 3010 York</b> <b>Oct 31<sup>st</sup> York 3010, 10:00 – 12, Oct 30<sup>th</sup> 9:30 – 11:30, Oct 30<sup>th</sup> 5:00 – 6:30</b>

		<p><b>How energy is produced – aerobic vs. anaerobic breakdown of organic compounds</b></p> <p>Acid and gas from sugar fermentation - complete</p> <p>Methyl-Red and Voges Proskauer – complete</p> <p>T-streak plate with unknowns for fresh isolated colonies (for Cyto C and catalase)</p> <ul style="list-style-type: none"> <li>• Oxygen requirements – inoculate thioglycolate tube</li> <li>• H<sub>2</sub>S production – inoculate Kligler iron deep</li> <li>• Nitrate reduction – inoculate nitrate broth</li> </ul> <p><b>Motility</b> – complete</p> <p>Start all unknown repeats</p> <p><b>Additional Lecture and Review: Tues sections, Room 3010 York</b></p>	
		<p><b>Students come in on non lab day to check thioglycolate tube and Kligler iron deep</b></p>	
Week 5 Lab 11	Thurs/Fri Nov 1/2	<p><b>How energy is produced – aerobic vs. anaerobic breakdown of organic compounds</b></p> <ul style="list-style-type: none"> <li>• Cytochrome C test – complete</li> <li>• Catalase test – complete</li> <li>• Oxygen requirements –complete</li> <li>• <b>Nitrate reduction</b> – complete</li> <li>• H<sub>2</sub>S production – complete test</li> <li>• Complete all unknown repeats</li> <li>• Extreme conditions optional tests</li> </ul>	<b>HW5 due- Dilution problems</b>
Week 6 Lab 12	Tues/Wed Nov 6/7	<p><b>Soil Enumeration and Enrichment:</b> First lab period:</p> <ul style="list-style-type: none"> <li>• <b>Enumeration:</b> Serial dilution, plating on TSA, SDA, GAA, and MacConkey, minimal media + skim</li> </ul>	<b>Quiz 6</b>

		<p>milk plates (Extracellular degradation of casein), TSA incubated at high temp (Extreme conditions: thermophiles) Incubation at assigned temperature</p> <ul style="list-style-type: none"> <li>• <b>Enrichment of soil organisms:</b> inoculation of MM = skim milk or TSB as assigned; incubation at assigned temperature</li> </ul> <p><b>Nitrogen fixation: Free-living - Anabaena</b> Inoculate BG11 and BG11-0 with <i>Anabaena</i></p> <p><b>Screening for Antibiotic Producers: day 1</b></p>	
		<p><b>Non lab day: TAs set up subculture of enrichments: Tues/Thurs TAs - Sat and Mon; Wed/Fri TAs – Sun and Tues</b></p>	
Week 6 Lab 13	Thurs/Fri Nov 8/9	<p><b>Soil Enumeration and Enrichment:</b> Second lab period</p> <ul style="list-style-type: none"> <li>• <b>Enumeration:</b> Colony counts and calculations</li> <li>• <b>Enrichment:</b> serial dilution and plating; centrifuge cell suspension and freeze pellet</li> </ul> <p><b>Screening for Antibiotic Producers: day 2</b></p> <p><b>Characterization of a Test Organism:</b> Create elimination flow chart for identification of genera</p>	<p><b>Midterm 2 in lab</b></p> <p><i>Computer lab – Begin elimination tree for HW 6- Identification of Unknown Organism– time permitting</i></p>
Week 7 Lab 14	Tues/Wed Nov 13/14	<p><b>Soil Enumeration and Enrichment:</b> Third lab period</p> <ul style="list-style-type: none"> <li>• <b>Enumeration:</b> Complete colony counts and calculations</li> <li>• <b>Enrichment:</b> Colony counts, examination for casein hydrolyzers, calculations</li> </ul> <p><b>Metagenomics:</b> First lab period</p> <ul style="list-style-type: none"> <li>• Discussion of metagenomics principles and options</li> </ul> <p><b>Characterization of a Test Organism:</b></p>	<p><b>Quiz 7</b></p> <p><b>Computer lab – complete elimination tree for HW 6 – Identification of Unknown Organism</b></p>

		Complete elimination flow chart for identification of genera	
Week 7 Lab 15	Thurs/Fri Nov 15/16	<b>Soil Enumeration and Enrichment:</b> Fourth lab period <ul style="list-style-type: none"> <li>• <b>Enrichment:</b> Complete colony counts, examination for casein hydrolyzers, calculations</li> </ul> <b>Evaluation of antibiotics by the Kirby Bauer method</b> Spread plates with standards and test efficiency of antibiotics	
Week 8 Lab 16	Tues/Wed Nov 20/21	<b>Evaluation of Antibiotics by the Kirby Bauer Method</b> Measure ZOI, identify any resistant colonies <b>Growth curve experiment</b> Growth and graphing of <i>Vibrio natriegens</i>	<b>HW 6 due – identification of unknown (40 points)</b> <b>HW 7 – Growth curve – begin work in lab, complete at home</b>
	Thurs/Fri Nov 22/23	<b>UCSD holiday for Thanksgiving</b>	
Week 9 Lab 17	Tues/Wed Nov 27/28	<b>Transposon mutagenesis:</b> Lab Period 1 Step 1: Set up conjugation of <i>E.coli</i> and <i>Salmonella</i> <b>Yogurt:</b> Inoculate milk with starter yogurt <b>Metagenomics:</b> Analysis of sample sequence data and sample construction of phylogenetic tree– computer lab	<b>Computer lab</b> <b>HW7 due – growth curve</b> <b>Quiz 8</b>
Week 9 Lab 18	Thurs/Fri Nov 29/30	<b>Transposon mutagenesis:</b> Lab Period 2 <ul style="list-style-type: none"> <li>• Step 2: Plate exconjugants for selection and counterselection</li> </ul> <b>Yogurt:</b> measure pH, gram stain <b>Metagenomics:</b> Step 8: Begin/complete analysis of all sequences, construction of phylogenetic trees. <b>Nitrogen Fixation</b> Free-living: <i>Anabaena</i> : check for heterocysts Symbiotic: <i>Rhizobium</i> : Observe nodules <b>Plant Pathogen</b> Observe <i>Agrobacterium</i> -kalanchoe	<b>Computer lab</b>

		interaction	
Week 10 Lab 19	Tues/Wed Dec 4/5	<b>Transposon mutagenesis:</b> Lab Period 3 <ul style="list-style-type: none"> <li>Count transconjugant colonies and calculate transposition efficiency</li> </ul> Lab clean up and check out	<b>Quiz 9</b> <b>HW8 due – Post workshop library survey</b> <b>Complete calculations for Lab Report</b>
Week 10 Lab 20	Thurs/Fri Dec 6/7	Midterm 3 will be held during regular lab hours	<b>Midterm 3</b>
	Mon Dec 10	<b>Mon Dec 10<sup>th</sup> 1 pm (Mon of finals week)</b> <b>– Lab Report due</b>	