

# **LABORATORY IN MICROBIOLOGY**

## **BIMM 121**

### **Fall 2009**

Mandatory Lecture: T/Th 12:30 – 1:50 am; Sequoyah Hall 147  
Lab: T/Th 2:00 pm – 6:00 pm; 2310, 2332 York Hall

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Week /Lab	Date	Experiment	Reports, Quizzes, Midterms, Reminders
Lab 1	Thurs 9/24	<ul style="list-style-type: none"> <li>Registration, introductory remarks, safety lecture, etc.</li> </ul> <b>Sterile technique.</b> <ul style="list-style-type: none"> <li>Expt 1: Microbes in the environment</li> <li>Expt 2: <i>E.coli</i> and toilet paper experiment</li> <li>Expt 3: Aseptic technique, streak and spread plates of a mixed culture for isolated colonies.</li> <li>Use of pipettors: Demo</li> </ul>	<b>Reminder: Discuss food samples for contamination</b>
Lab 2	Tues 9/29	<b>Sterile technique.</b> <ul style="list-style-type: none"> <li>Expt 1: Microorganisms in the environment: Observe results</li> <li>Expt 2: <i>E.coli</i> and toilet paper experiment: Observe results</li> <li>Expt 3: Streak and spread plates: Observe results.</li> </ul> <b>Microscopy:</b> <ul style="list-style-type: none"> <li>Expt 4A: Learning to focus the light microscope</li> <li>Expt 4B: Calibrating your microscope</li> <li>Expt 4C: Observing live microorganisms: The wet-mount and phase-contrast microscopy               <ul style="list-style-type: none"> <li>Bright-field vs. phase-contrast microscopy</li> <li>Prokaryotes vs. Eukaryotes</li> </ul> </li> </ul> <b>Plant Pathogens:</b> <ul style="list-style-type: none"> <li>Expt 34: Set up <i>Agrobacterium</i>-kalanchoe infections</li> </ul> <b>Winogradsky column</b> <ul style="list-style-type: none"> <li>Understanding the set up</li> </ul>	<b>Reminder: Set up food samples for contamination</b>
Lab 3	Thurs 10/1	<b>Understanding dilutions:</b> <ul style="list-style-type: none"> <li>Expt 5A: Understanding dilutions- theory</li> </ul>	<b>Daily quiz 1</b> <b>REMINDER: Take food</b>

		<b>Measuring Microbial Growth: Yeast and SDA</b> <ul style="list-style-type: none"> <li>○ Expt 6A: Using a spectrophotometer</li> <li>○ Expt 6B: Use of a hemocytometer</li> <li>○ Expt 6C: Counting viable cells using plating</li> </ul> <b>Microscopy: Staining</b> <ul style="list-style-type: none"> <li>○ Expt 7A&amp;B: Smear preparation and simple staining</li> <li>○ Expt 7C: Gram stain: standard organisms only</li> </ul>	<b>samples home today and bring back on Tuesday</b>
Lab 4	Tues 10/6	<b>Microscopy: Staining</b> <ul style="list-style-type: none"> <li>○ Repeat staining and microscopy as required</li> </ul> <b>Growth curve experiment</b> <ul style="list-style-type: none"> <li>○ Expt 8: Growth and graphing of <i>Vibrio natriegens</i></li> </ul> <b>Detection of bacterial food contaminants</b> <ul style="list-style-type: none"> <li>○ Expt 9: Serial dilution and plating</li> </ul> <b>Unknown Organism</b> <ul style="list-style-type: none"> <li>○ Expt 10A: Receive unknown: wet mount and streak plate for single colonies.</li> </ul>	<b>Daily quiz 2 REMINDER: Bring contaminated food samples for experiment today</b>
Lab 5	Thurs 10/8	<b>Unknown Organism:</b> <ul style="list-style-type: none"> <li>○ Expt 10A: Examine streak plate</li> <li>○ Expt 10B: Inoculate broths, slants and plates with unknown</li> <li>○ Gram stain of unknown organism</li> <li>○ First microscopic examination of unknown: wet mount for shape, size, and motility</li> <li>○ Expt 13: Nutrient Sporulation Medium (NSM) – streak slant</li> </ul> <b>Isolation of bacterial food contaminants:</b> <ul style="list-style-type: none"> <li>○ Expt 9: Complete colony counts</li> </ul> <b>TA-run workshop on writing a report</b>	<b>Daily quiz 3</b>

Lab 6	Tues 10/13	<b>Unknown Organism:</b> <ul style="list-style-type: none"> <li>○ Expt 11: Streak unknown on MacConkey plate</li> <li>○ Bacterial motility <ul style="list-style-type: none"> <li>• Expt 12A: Preparing wet mounts</li> <li>• Expt 12B: Observing motility on plates, deeps - inoculate</li> </ul> </li> <li>○ Expt 13: Bacterial endospores <ul style="list-style-type: none"> <li>• NSM: Wet mount (and simple stain)</li> </ul> </li> <li>○ Expt 14: Nitrate reduction – inoculate</li> <li>○ Expt 15: Oxygen requirements – inoculate thioglycolate tube</li> </ul>	<b>Midterm 1: Topics as posted on class board</b>
Lab 7	Thurs 10/15	<b>Unknown organism:</b> <ul style="list-style-type: none"> <li>○ Expt 11: Examine MacConkey</li> <li>○ Expt 12: Motility – complete</li> <li>○ Expt 14: Nitrate reduction - complete</li> <li>○ Expt 15: Oxygen requirements – complete</li> <li>○ Expt 16: H<sub>2</sub>S production – inoculate</li> <li>○ Expt 17: Acid and gas production from sugar fermentation – inoculate fermentation tubes</li> <li>○ Expt 18: Methyl-Red and Voges-Proskauer – inoculate</li> <li>○ Expt 19A&amp;B: Streak plate with unknown</li> </ul>	<b>Report 1 Due Today: Bacterial contamination of food</b>
Lab 8	Tues 10/20	<b>Unknown organism:</b> <ul style="list-style-type: none"> <li>○ Expt 16: H<sub>2</sub>S production - Check, reincubate as necessary</li> <li>○ Expt 17: Acid and gas from sugar fermentation - complete</li> <li>○ Expt 18: Methyl-Red and Voges Proskauer – complete</li> <li>○ Expt 19A: Cytochrome C test – complete</li> <li>○ Expt 19B: Catalase test – complete</li> <li>○ Hydrolysis and use of large extracellular materials – inoculate</li> </ul>	<b>Daily quiz 4</b>  <b>Reminder: Bring water sample on Thurs– 1 sample per student, ~75 ml in any clean container (Streak plate test today)</b>

		<ul style="list-style-type: none"> <li>Expt 20A: Polysaccharides: Starch plates</li> <li>Expt 20B&amp;C: Proteins: Skim milk plates and gelatin deeps</li> <li>Expt 20D: Lipids: Rhodamine plates</li> </ul>	
Lab 9	Thurs 10/22	<p><b>Unknown organism:</b></p> <ul style="list-style-type: none"> <li>Expt 20: Hydrolysis and use of large extracellular materials - complete</li> <li>Expt 21: Indole production from tryptophan, catabolite repression – inoculate</li> <li>Expt 22: Urease test – inoculate</li> <li>Expt 23: Differential utilization of citrate by enterics - inoculate</li> </ul> <p><b>Coliforms in water</b></p> <ul style="list-style-type: none"> <li>Expt 24: Colilert, incubation of water sample</li> </ul>	<p><b>Daily quiz 5</b></p> <p><b>Reminder: Bring water sample today.</b></p>
Lab 10	Tues 10/27	<p><b>Unknown organism:</b></p> <ul style="list-style-type: none"> <li>Expt 21: Indole production from tryptophan, catabolite repression - complete</li> <li>Expt 22: Urease test - complete</li> <li>Expt 23: Differential utilization of citrate by enterics - complete</li> <li>Unknown Repeats</li> </ul> <p><b>Coliforms in water</b></p> <p>Expt 24: Examine Colilert and set up Levine EMB</p> <p><b>Transposon mutagenesis</b></p> <ul style="list-style-type: none"> <li>Expt 25A: Set up conjugation</li> </ul> <p><b>Evaluation of antiseptics and disinfectants</b></p> <p>Expt 26: Spread plates with standards and test efficiency of antiseptics and disinfectants</p>	<p><b>Daily quiz 6</b></p> <p><b>Reminder: TAs bring soil sample for next lab</b></p>
Lab 11	Thurs 10/29	<p><b>Unknown organism</b></p> <ul style="list-style-type: none"> <li>Complete repeats</li> </ul> <p><b>Coliforms in water</b></p>	<p><b>Daily quiz 7</b></p> <p><b>Syllogistic tree for</b></p>



		<p>Expt 24: Set up Enterotube</p> <p><b>Transposon mutagenesis</b></p> <ul style="list-style-type: none"> <li>Expt 25A: Set up selection</li> </ul> <p><b>Evaluation of antiseptics and disinfectants</b></p> <p>Expt 26: complete</p> <p><b>Soil Day 1:</b></p> <ul style="list-style-type: none"> <li>Expt 27A : Serial dilution, plating on TSA, SDA, GAA, and MacConkey – aerobic and anaerobic</li> <li>Expt 27C: Plate on differential media</li> <li>Expt 27D: Inoculate enrichment media flasks</li> <li>Expt 27F: Begin testing for presence of spores (exposure to high heat, serial dilution, and plating)</li> </ul>	<p><b>“Unknown” report due</b></p> <p><b>Reminder: TAs bring soil sample today</b></p>
Lab 12	Tues 11/3	<p><b>Coliforms in water</b></p> <p>Expt 24: Evaluate Enterotube</p> <p><b>Transposon mutagenesis</b></p> <ul style="list-style-type: none"> <li>Expt 25B: Identify and streak out pigment mutants</li> <li>Expt 25C: Grid to select for lipase, amino acid auxotroph, and other mutants</li> </ul> <p><b>Soil Day 2:</b></p> <ul style="list-style-type: none"> <li>Expt 27B: Soil metagenomics: <ul style="list-style-type: none"> <li>Total DNA preparation</li> <li>Set up 16S rRNA PCR reaction</li> </ul> </li> <li>Expt 27F: Count colonies</li> </ul>	<p><b>Midterm 2: Topics as posted in class</b></p>
Lab 13	Thurs 11/5	<p><b>Transposon mutagenesis</b></p> <ul style="list-style-type: none"> <li>Expt 25D: Final selection of mutants, streak for PCR</li> </ul> <p><b>Soil Day 3:</b></p> <ul style="list-style-type: none"> <li>Expt 27A: Enumeration: colony counts</li> </ul>	<p><b>Daily quiz 8</b></p>

		<ul style="list-style-type: none"> <li>○ Expt 27B: Metagenomics: Purification of PCR product</li> <li>○ Expt 27C: Examine and enumerate colonies on differential media</li> <li>○ Expt 27D: Subculture enrichment flasks</li> <li>○ Expt 27E: Identification of antibiotic producers: Grid plates</li> </ul> <b>Nitrogen Fixation - Anabaena</b> <ul style="list-style-type: none"> <li>○ Expt 28A: Inoculate BG11 and BG11-0</li> </ul>	
Non lab day		<b>TAs set up PCR of transposon mutants</b>	
Lab 14	Tues 11/10	<b>Transposon mutagenesis</b> <ul style="list-style-type: none"> <li>○ Expt 25D: PCR product purification</li> <li>○ Run gel and send product for sequencing</li> </ul> <b>Computer lab:</b> <ul style="list-style-type: none"> <li>○ Analysis of sample sequence.</li> <li>○ Using the BIOCYC website</li> </ul> <b>Soil Day 4:</b> <ul style="list-style-type: none"> <li>○ Expt 27B: Metagenomics: Clone PCR product into pGEM-T, transform <i>E.coli</i></li> <li>○ Expt 27E: Identification of antibiotic producers: check for ZOI, select and set up for colony PCR (16s rRNA), inoculate broth for possible antibiotic production</li> </ul>	<b>Report 2A due</b>
Lab 15	Thurs 11/12	<b>Transposon mutagenesis</b> <ul style="list-style-type: none"> <li>○ Expt 25D: Analyze sequence?</li> <li>○ Expt 25E: Set up complementation of auxotrophs with amino acids?</li> <li>○ Tabulate all information on color, site of mutation and non-pigment mutants?</li> </ul> <b>Soil Day 5:</b> <ul style="list-style-type: none"> <li>○ Expt 27A: Wet mounts</li> </ul>	<b>Daily quiz 9 (sterile technique test)</b>

		<ul style="list-style-type: none"> <li>○ Expt 27B: Metagenomics: Restreak colonies for sequencing</li> <li>○ Expt 27D: Plate from flasks onto enrichment plates; Plate dilutions onto SDA, GAA, and TSA plates</li> <li>○ Expt 27E: Identification of antibiotic producers: Purification of PCR product, send for sequencing; Test putative antibiotic produced by antibiotic producer</li> </ul> <p><b>UV mutagenesis</b> Expt 29: Start experiment</p> <p><b>Evaluation of antibiotics by the Kirby Bauer method</b></p> <ul style="list-style-type: none"> <li>○ Expt 30: Spread plates with standards and test efficiency of antibiotics</li> <li>○ <i>Test putative antibiotic produced by antibiotic producer</i></li> </ul>	
	Non-lab day	<p><b>Metagenomics and Antibiotic Producers</b> Send streak plates and PCR products for sequencing</p>	
Lab 16	Tues 11/17	<p><b>Transposon mutagenesis</b></p> <ul style="list-style-type: none"> <li>○ Expt 25D: Analyze sequence</li> <li>○ Expt 25E: Set up complementation of auxotrophs with amino acids</li> <li>○ Tabulate all information on color, site of mutation and non-pigment mutants</li> </ul> <p><b>Soil Day 6:</b></p> <ul style="list-style-type: none"> <li>○ Expt 27D: Examine enrichment plates and SDA, GAA, TSA plates</li> <li>○ Expt 27E: Antibiotic Producers: Computer Lab (if sequences are ready)</li> </ul> <p><b>UV mutagenesis</b> Expt 29: Observe plates</p> <p><b>Evaluation of antibiotics</b></p> <ul style="list-style-type: none"> <li>• Expt 30: measure ZOI, identify any resistant colonies, set up broth cultures of resistant colonies</li> </ul> <p><b>Bacterial viruses</b></p> <ul style="list-style-type: none"> <li>○ Expt 31: Start infection (TAs). Serial dilution and plating using soft-</li> </ul>	<p><b>Daily quiz 10</b> <b>Report 2B due</b></p> <p><b>Reminder: Take home saliva collection tube today</b></p>

		overlay method	
Non lab day		Sequences posted over the weekend (start analysis)	
Lab 17	Thurs 11/19	<b>Transposon mutagenesis</b> <ul style="list-style-type: none"> <li>Expt 25E: Observe results of complementation</li> </ul> <b>Soil Day 7:</b> <ul style="list-style-type: none"> <li>Expt 27B: Metagenomics: Computer Lab</li> <li>Expt 27E: Antibiotic Producers: Computer Lab</li> </ul> <b>Nitrogen Fixation</b> <ul style="list-style-type: none"> <li>Expt 28A: <i>Anabaena</i> – examine for heterocysts</li> <li>Expt 28B: Observe <i>Rhizobium</i>-bean interaction.</li> <li>Culture <i>Rhizobium</i> from roots</li> <li>Measure nitrogen</li> </ul> <b>Evaluation of antibiotics</b> Expt 30: test resistance of resistant colonies <b>Bacterial viruses</b> <ul style="list-style-type: none"> <li>Expt 31: Enumerate viral plaques</li> </ul> <b>Dental Flora</b> <ul style="list-style-type: none"> <li>Expt 33: inoculate Snyder agar</li> </ul> <b>Yogurt Production</b> <ul style="list-style-type: none"> <li>Expt 34: inoculate milk</li> </ul>	<b>Reminder: Bring saliva sample today</b>
Lab 18	Tues 11/24	<b>Evaluation of antibiotics</b> Expt 30: observe results of resistance verification experiment <b>Nitrogen Fixation</b> <ul style="list-style-type: none"> <li>Expt 27A: <i>Anabaena</i>: check for heterocysts</li> <li>Expt 27B: <i>Rhizobium</i>: Observe TSA plates</li> </ul>	<b>Report 3 due</b>

		<b>Dental Flora</b> ○ Expt 32: complete <b>Yogurt Production</b> ○ Expt 33: complete <b>Plant Pathogen</b> Expt 34: Observe <i>Agrobacterium</i> -kalanchoe interaction	
Lab 19	Tues 12/1	Discussions/Presentations Lab clean up Potluck	
Lab 20	Thurs 12/3	Midterm 3 will be held during normal lab hours. No lecture today	<b>Midterm 3: Topics as posted in class.</b>

## **Class Policies:**

### **Equipment:**

For this lab you will need to purchase:

- A lab notebook (check with instructor to determine if notebook with carbons is required),
- A lab coat
- Eye protection (You may wear either safety glasses or goggles, but standard eye glasses are not sufficient.)
- 

### **Absences:**

1. 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.
2. Documentation will be required for all unavoidable absences.
3. If you are likely to have interviews for graduate school, etc, please schedule them on non-lab days.
4. All absences without prior notification/permission and the appropriate paperwork will be considered unauthorized.
5. **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.
6. 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.

### **Assignment Deadlines:**

1. All reports will be due at the beginning of lecture on the date indicated. Reports 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. 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.

## **Lab Performance and Participation**

Subjective student evaluations will be based on the following criteria:

- 1.** At least two lab techniques will be evaluated in class.
- 2.** Lab notebook
- 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.**

## **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 his 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.
- 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.

## **Grading Scheme**

<b>Quiz/Report/Midterm</b>	<b>Points</b>
Daily quizzes @ 5 points each	50
Lab techniques	10
Assignments	20
3 x midterms (40, 65, 85 pts or 40, 60, 90 pts)	190
3 x Reports (50, 100, 50 pts)	200
Lab performance, notebook, and participation	20
Presentation	<u>10</u>
<b>Total</b>	<b>500</b>