BIMM 121 Laboratory in Microbiology
Winter 2015

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***On Ted, in folder labeled "Useful Information"**

Lecture: Tuesday/Thursday 8:00 – 9:20 in 120 PCYNH (Pepper Canyon Hall; near the Gilman Parking Structure).

Labs: York 2310 and 2332
Tuesday/Thursday: 9:30 am – 1:30 pm

Office hours: Mondays 10:00 am-11:00 am. Location: 4070C York Hall or 2300 York Hall – to be confirmed.

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 two main units: a comprehensive look at bacterial physiology 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.
8. You need to inform both the TA and the instructor of any proposed absence. Only the instructor can decide whether or not the reason for an absence is sufficient to call it an authorized absence.

Reading for the lab

Reading ahead of the course:
If you wish to read ahead, your best bet is to brush up on any information on microbes, cell structure, and basic microbial physiology (glycolysis, TCA cycle, electron transport chain, central dogma, etc). All information pertinent to topics covered in class is in the manual and will be discussed in lecture. By the first lecture, I strongly recommend that you have at least some familiarity with the following material (that we anticipate you SHOULD know from your prerequisite courses):

- Definition of microbes and an understanding of the different groups of microbes (e.g. bacteria, fungi). You are not required to memorize all the names – you should, however, have at least a basic idea as to the types of organism included in each category.
- Understanding of the scientific method – brush up on this concept – there are several online sites, including Wikipedia, that do a good job of explaining dependent, independent, and controlled variables as well as the difference between a control experiment and a regular experiment.
- Understand how to make figures from Excel spreadsheets – how to calculate simpler values such as standard deviation, averages, etc and how to plot them as line/plot graphs with error bars.

Reading during the course:

- Read the chapters before you come to lecture. After week 1, I will post guidelines to reading the chapters in the folder labeled “Directed Reading” on TED
- When you are in the classroom, I will go over the basics as required, any fundamental concepts that you do find or might find difficult, that are important, or that are particularly exciting or newsworthy.
- Then you will go to lab and actually see all those tests and concepts in action.

- Then go back and quickly reread the material in light of the lecture and lab work and you will find that it becomes very clear since you are already familiar with most of it.

As often as possible, I will give you questions/problems to think about that should apply the concepts you learned in class. Thinking about and attempting to answer these questions and participating in any classroom/lab discussion is the best practice you can have for midterms, lab reports, and practicing science in general.

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:

1. Pre-lab preparation
2. Careful management of lab procedures (e.g., sterile technique, proper waste disposal, experimental procedures, etc.)
3. Ability to adapt to unforeseen procedural changes
4. Caliber of thinking before asking questions
5. Scientific approach (e.g., proper use of notebooks, controls, experimental design)
6. Accuracy
7. Independence
8. Safety consciousness
9. 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.**

CLICKERS in Micro Lab

This lab will introduce you to new material and concepts. To increase the depth of your understanding and to give you practice in applying these concepts, we will discuss these concepts from different perspectives in class. Over the last few

years, student feedback has shown that class participation has a very positive impact on performance in lab reports and midterms.

We will be using Clickers in class as part of the learning process and to help students stay on top of the concepts and their applications. Participation in the lecture discussion is worth 5% of your grade and requires that you click in at least 75% of the time in each lecture for at least 75% of the lectures. i>Clickers are available for purchase at the UCSD bookstore. Once you have purchased your Clicker, you can register it on Ted. A separate explanation of our Clicker policy is on Ted.

Notebook:

A 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.

Homework and Lab report Deadlines and Submission:

1. A hard copy of each homework is due in the first 5 minutes of the lab period of the day on which your report is due. **All homework assignments submitted more than 10 minutes after start of lab are automatically late and lose 10% of the points. Any homework submitted the next calendar day would lose 50% of the points. No homework will be accepted after the second calendar day.**
2. There is only one lab proposal and it is due the Mon of finals week. Any lab proposal turned in one day late will lose 50% of the points. Any lab proposal turned in more than one day late will not be graded.
3. In addition to the hard copy of the assignments/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 results 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
4. Additional points may be taken for late electronic submissions.

HW#	Description	Due date	Points
1	Library tutorial	Tues Jan 13	30
2	Scientific Method	Tues Jan 20	30
3	Growth curve	Thurs Jan 29	15
4	Dilution problems	Thurs Feb 5	20
5	Unknown analysis	Tues Mar 3	85
Total			**180**

Other important dates:

MT1 (in lecture): Tues Jan 27th
MT2 (in lecture and/or lab): Thurs Feb 19th
MT2 math: Tues Feb 24th
MT3 (in lab): Thurs Mar 13th
End of quarter concept analysis paper (HW6): Mon Mar 16th

Quizzes

Quiz 1: Thurs Jan 8th
Quiz 2: Thurs Jan 15th
Quiz 3: Thurs Jan 22nd
Quiz 4: Thurs Feb 5th
Quiz 5 Thurs Feb 12th
Quiz 6: Thurs Feb 26th
Extra quiz: Date to be determined

Grading Scheme

Evaluation criterion	Points	% of total
Competency	57	7.1
Lab notebook	36	4.5
Clicker	42	5.3
Homework	180	22.5
Quizzes	90	11.3
Midterms	335	41.9
End of quarter concept analysis paper (HW6)	60	11.3
Total Possible	**800**	

Most Likely Grade Distribution

A = 90% - 100%

B = 80% - 89.9%

C = 70% - 79.9%

D = 60% - 69.9%

F = below 60%

Regrade Requests:

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

Course Website/Ted

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 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.
- **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.

Week 1

Lab 1: **Tues/Wed Jan 6/7**	**Lab 2**: **Thurs/Fri Jan 8/9**
Registration, attendance, safety video, integrity agreement, responsibility agreement, introductory remarks, safety lecture **Aseptic technique.** • Microbes in the environment • Why wash your hands? **Use of pipettors:** Demo and exercise Possible pre-course survey	**Aseptic technique.** Microbes in the environment: Observe results *E.coli* and toilet paper experiment: Observe results Aseptic technique: streak and spread plates • Demo • Lab exercise using a mixed bacterial culture **Microscopy:** Learning to focus the light microscope • Demo • Lab exercise using prepared (commercial) slides • Learning to draw Cleaning your microscope – demo and completion

Week 2

Lab 3: Tues/Wed Jan 13/14	Lab 4: Thurs/Fri Jan 15/16
Aseptic technique. Aseptic technique: streak and spread plates Observe results **Characterizing the Unknown Organisms:** Receive unknown organisms and inoculate one Trypticase Soy slant (TSS) TAs will incubate at appropriate temperature **Microscopy:** Calibrating your microscope: Demo and complete Complete examination of prepared slides Evaluation of recorded drawings **Selective and Differential media**: an introduction Coliforms in water; Levine EMB **HW1 due: Scientific method**	**Microscopy: Staining** • Demo of smear • Smear preparation and simple staining • Gram stain: Complete staining of designated Gram-positive and Gram-negative controls • Gram staining of Unknown from TSS prepared on lab 3.

Week 3

Lab 5: Tues/Wed Jan 20/21	Lab 6: Thurs/Fri Jan 22/23
Microscopy: Wet mounts and phase contrast: view, identify, and measure (Listed Eukaryotes, Bacteria, Mixed cultures, and Hay Infusion) **Characterizing the Unknown Organisms: Wet mount and Temperature Preference** Use **original** slant of unknown organisms • make a wet mount, • inoculate streak plates and broth cultures for temperature preference **Understanding dilutions:** Understanding dilutions- theory only **HW 2 due: Online library tutorial**	**Measuring microbial growth: Yeast** • Direct counts using a hemocytometer • Using a spectrophotometer • Counting viable cells using plating **Characterization of the Unknown Organisms** Confirm temperature preference • Observation of streak plates • OD measurements Do wet mounts from both temperatures Streak stock TSS with unknown, incubate at optimum temperature

Week 4

Lab 7: Tues/Wed Jan 27/28 **MT 1: in lecture** **Growth curve experiment** Growth and graphing of *Vibrio natriegens* **Characterization of the Unknown Organisms** MacConkey – inoculate along with known G+ and G- organisms Sticky test, along with known G+ and G- organisms CONFIRM GRAM RESULT TODAY! BEGIN GENUS CHARTS TODAY! (Assign genera to members of group)	**Lab 8**: **Thurs/Fri Jan 29/30** **Macronutrient use – how organisms get energy to survive** • Introduction: Hydrolysis and use of large extracellular materials • Polysaccharides: Starch plates - inoculate • Proteins: Skim milk plates and gelatin deeps – inoculate • Lipids: Rhodamine plates – inoculate **Scientific Method/Scientific literacy/HW6 Workshop: Last 3 hours of lab day** **HW3 due: Growth curve** **Computer lab 3060 and 3070 York Hall**

Week 5

Lab 9: Tues/Wed Feb 3/4	Lab 10: Thurs/Fri Feb 5/6
Macronutrient use – how organisms get energy to survive • Polysaccharides: Starch plates - complete • Proteins: Skim milk plates and gelatin deeps - complete • Lipids: Rhodamine plates – complete **Endospore test** – inoculate NSM **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Acid and gas from sugar fermentation – inoculate • Methyl-Red and Voges-Proskauer – inoculate **Inoculate fresh stocks of unknowns and control organisms:** 1. Unknowns (T-streak) 2. *Escherichia coli* 3. *Pseudomonas fluorescens* 4. *Enterococcus faecalis* 5. *Staphylococcus epidermidis* 6. *Pseudomonas aeruginosa* Inoculate 1 TSS or TSA of each **control** per aisle. These stocks will be used for the nitrate, Cyto C and catalase tests in Lab 10 **DISCUSSION/LEARNING TIME – 60 minutes**	**How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Acid and gas from sugar fermentation - complete • Methyl-Red and Voges Proskauer – complete • Cytochrome C test – complete • Catalase test – complete • Nitrate reduction – inoculate nitrate broth **Endospore test**: NSM – Complete – microscopy **DISCUSSION/LEARNING TIME – 60 minutes** **HW4 due: Dilution problems**

Week 6

Lab 11: Tues/Wed Feb 10/11	Lab 12: Thurs/Fri Feb 12/13
Characterization of the Unknown Organisms: **Motility** – inoculate plate and deep with unknowns and controls **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • H_2S production – inoculate Kligler iron deep • Oxygen requirements – inoculate thioglycolate tube • **Nitrate reduction** – complete **Inoculate fresh stocks for urease test in lab 12** *1. Enterobacter aerogenes* 2. *Proteus vulgaris* 3. Your unknown **4. *TAs inoculate E. coli*** Inoculate 1 TSS or TSA of each **control** per aisle. **DISCUSSION/LEARNING TIME – 2 hours (Suggested Kligler, thioglycolate, nitrate)** **Students come in on non lab day to check thioglycolate tube and Kligler iron deep**	**Characterization of the Unknown Organisms:** **Motility** – complete • Observe plates and deeps • Observe wet mounts of controls and unknowns **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Oxygen requirements –complete • H_2S production – complete **Special metabolic functions:** • Indole production from tryptophan, catabolite repression – inoculate • Urease test – inoculate • Differential utilization of citrate by enterics – inoculate **Complete all genus charts** **DISCUSSION/LEARNING TIME – 60– 90 min. Begin proposal work.**

Week 7

Lab 13: Tues/Wed Feb 17/18	**Lab 14: Thurs/Fri Feb 19/20**
Characterization of the Unknown Organisms: **Special metabolic functions** • Indole production from tryptophan, catabolite repression – complete • Urease test – complete • Differential utilization of citrate by enterics – complete Begin any repeat tests **Extreme conditions** Check tolerance of unknown for low/high pH, different temperatures, salinity, using media provided - inoculate **Nitrogen fixation: Free-living - Anabaena** TAs inoculate BG11 and BG11-0 with *Anabaena* **TAs check all genus charts**	**Midterm 2 in lab or lecture** **Characterization of the Unknown Organisms:** Complete all repeated tests **Extreme conditions:** Record results **DISCUSSION/LEARNING TIME – 60– 90 minutes in computer lab: Begin HW6 development**

Week 8

Lab 15: Tues/Wed Feb 23/24	Lab 16: Thurs/Fri Feb 25/26
Antibiotic Producer Spread plates and grid out antibiotic producers and non-producers **Characterization of the Unknown Organisms:** Create elimination flow chart for identification of genus and species – computer lab	**Antibiotic Producer** Evaluate Zones of Inhibition **Nitrogen Fixation** 1. Free-living: *Anabaena*: check for heterocysts ***2.*** Symbiotic*: Rhizobium:* Observe nodules **Evaluation of antibiotics by the Kirby Bauer method** Spread plates with standards and test efficiency of antibiotics **Yogurt:** Investigate each type of milk, discuss possible hypotheses. Group of 4 will formulate hypothesis and design experiment for next lab. Measure pH, glucose, protein in uninoculated milk, sample yogurt, buttermilk, kefir

Week 9

Lab 17: Tues/Wed Mar 3/4	**Lab 18: Thurs/Fri Mar 5/6**
Transposon mutagenesis: Lab Period 1 Step 1: Set up conjugation of *E.coli* and *Salmonella* **Evaluation of Antibiotics by the Kirby Bauer Method** Measure ZOI, identify any resistant colonies **Yogurt:** Check hypothesis and experiment design with TA/Instructor Inoculate control and experimental milk with starter culture and incubate under desired conditions Gram stain dairy products provided Check pH, glucose, protein, and thickness at start and 3 hour time point Inoculate a fresh culture of *Staphylococcus* to use as a Gram+ control in staining, lab 18. **HW5 due: Unknown organism**	**Transposon mutagenesis:** Lab Period 2 • Step 2: Plate exconjugants for selection and counterselection Save LB recipient control plates for later use **Yogurt:** • Measure pH, glucose, and protein. • Gram stain. • Collate information with lab partners **DISCUSSION/LEARNING TIME/HW6 in-class work**

Week 10

Lab 19: Tues/Wed Mar 10/11	**Lab 20: Thurs/Fri Mar 12/13**
Transposon mutagenesis: Lab Period 3 • Count colonies and calculate transposition efficiency Lab clean up and check out **Wrap and label lab coats for autoclaving** **Yogurt: Discussion of collated data.** **Computer lab: HW6 development**	**Midterm 3** will be held in lab during regular lab hours

Finals Week

Mon/Tue Mar 16/17
Mon, Tues of finals week - 1 pm – HW6 due.