BIMM 121 Laboratory in Microbiology
Spring 2017

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

Lecture: Tuesday/Thursday 8:00 – 9:20 am, **Center 109 – note change in venue.**

Labs: York 2310 and 2332
Tues/Thurs: 9:30 am – 1:30 pm
Wed/Fri: 9:00 am – 1:00 pm

Office hours: Mondays 11:00 am – 12:00 noon. Location: 2300 York Hall. Office hours will be conducted as mini reviews and open format discussion/question sessions.

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 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 (safety glasses preferred, standard prescription eye glasses are not sufficient).
- A Sharpie permanent marker pen, preferably fine point (not extra fine or regular). Black or blue. Avoid red and light colors.

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 IA 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:
I will assume that you all have a basic understanding of, and reasonably good memory of the following from lower division bio or form high school. If you don't remember, you may wish to read ahead:

- 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.
- 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
- Eukaryotic vs. prokaryotic cells structure.

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.

A sample notebook page will be posted on TritonEd.

Homework and Lab report Deadlines and Submission:

1. A hard copy of your 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/Tues of finals week for T/Th and W/F sections respectively. 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 may be 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**
Extra Credit	Pre course safety survey	Thurs April 6	(3)
Day 1 activity	Scientific Method	Thurs/Fri April 4	20
1	Library tutorial	Tues/Wed April 11/12	25
2	Simple dilutions	Tues/Wed 18/19	20
3	Growth curve	Tues/Wed May 2/3	35
4	Complex dilutions	Tues/Wed May 9/10	25
5	Unknown analysis	Tues/Wed May 30/31	100
Extra Credit	Post course safety survey	Wed June 6	(3)
End of Quarter: Concept analysis paper		Mon/Tues June 12/13	80
Total			**305**

Other important dates:

MT1 (in lecture): Tues April 25th
MT2 (in lecture): Tues May18th
MT3 (in lab): Thurs/Fri June 8th/9th
End of quarter concept analysis paper (HW6): Mon/Tues June 12th/13th

Quizzes

Quiz 1: Thurs/Fri April 6th/7th
Quiz 2: Thurs/Fri April 13th/14th
Quiz 3: Thurs/Fri April 20th/21st
Quiz 4: Thurs/Fri May 4th/5th
Quiz 5: Thurs/Fri May 11th/12th
Quiz 6: Thurs/Fri May 25th/26th
Extra quiz: Thurs/Fri June 1st/2nd

Quizzes begin on the first Thurs/Fri of the quarter. Quizzes will be held in the first 10-15 min of lab. Please come on time since you will not be given extra time if you are late. We will have 6 quizzes each worth 18 points for a total of 108 points. An extra quiz will be offered to make up for any missed quizzes since there will be no make up quizzes. Students who have already taken all 6 quizzes may also choose to take the extra quiz and drop the lowest score of the 7 total quizzes.

Grading Scheme

Evaluation criterion	**Points**	**% of total**
Competency	70	7.0
Lab notebook	42	4.2
Clicker	56	5.6
Homework	305	30.5
Quizzes	108	10.8
Midterms	419	41.9
Total Possible	**1000**	**100. 0**

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. 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: **Tue/Wed April 4/5**	**Lab 2**: **Thu/Fri April 6/7**
Assigned before class: DOB safety training – mandatory BIMM 121 Pre-course survey Scientific method - read _____ Safety video Registration, attendance Integrity agreement Responsibility agreement, introductory remarks, safety lecture **Begin:** **Sterile technique** • Swab plating of mixed culture on TSA, MacConkey, Blood agar **Begin & Complete:** **Use of pipettors:** Demo and exercise Scientific Method in-class work Pre-course survey due by 8 am Online safety by 8 am Scientific Method in-class graded work due in class (30 points)	**Assigned before class:** Aseptic technique video _____ **Complete:** **Sterile technique.** Mixed culture: Observe results **Begin** Sterile technique: streak and spread plates • Demo • Lab exercise using a mixed bacterial culture **Begin & Complete:** **Microscopy:** Learning to focus the light microscope • Demo and Video: parts, focusing, cleaning: • Microscopy and learning to draw using prepared (commercial) slides • Cleaning your microscope and Microscope checks Quiz 1

Week 2

Lab 3: Tue/Wed April 11/12

Assigned before class:

Calibration video

Complete:

Sterile technique

Sterile technique: streak and spread plates

Observe results: self- and peer-evaluation

Begin & Complete:

Microscopy:

Calibrating your microscope: Demo and complete

Complete examination of prepared slides

Evaluation of recorded drawings

Microscope clean up and check

Begin:

Characterizing the Unknown Organisms:

Receive unknown organisms and inoculate one Trypticase Soy slant (TSS)

IAs will incubate at appropriate temperature

Selective and Differential media - Colilert and Levine EMB

HW1: Library tutorial

Lab 4: Thu/Fri **April 13/14**

No assigned videos

Begin & Complete:

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.

Begin:

Endospore test

Inoculate:

NSM – controls only

Quiz 2

Week 3

Lab 5: Tue/Wed April 18/19

Assign before class:
Dilutions videos:

- Terms and definitions
- Simple
- Mixture

Begin & Complete:
Microscopy
Video: Phase contrast microscopy
Wet mounts and phase contrast: view, identify, and measure (Listed Eukaryotes, Bacteria, Mixed cultures)

Complete:

Endospore test

NSM –phase contrast microscopy and simple stain of controls only

Begin:
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

Microscope clean up and check
HW 2: Simple dilution math

Lab 6: Thu/Fri April 20/21

Assign before class:
Dilutions videos:

- Multistep series
- Multistep serial
- Working with microbes

Complete:

Characterization of the Unknown Organisms

Confirm temperature preference

- Observation of streak plates
- OD measurements

Do wet mounts from both temperatures -

Streak unknown into a TSS slant

Worksheets provided

Begin & Complete:
Measuring microbial growth: Yeast

- Direct counts using a hemocytometer
- Using a spectrophotometer
- Counting viable cells using plating

Microscope clean up and check
Quiz 3

Week 4

Lab 7: Tue/Wed April 25/26

MT 1: in lecture

Complete:

Characterization of the Unknown Organisms

Sticky test, along with known G+ and G- organisms

CONFIRM GRAM RESULT TODAY!

Begin & Complete:

Growth curve experiment

Growth and graphing of *Vibrio natriegens*

Begin:

Characterization of the Unknown Organisms

Inoculate:

- MacConkey – along with known G+ and G- organisms

BEGIN GENUS CHARTS TODAY!

(Assign genera to members of group)

NOTE: inoculations, tests, and charts can be done DURING growth curve

Lab 8: Thu/Fri April 27/28

Assign before class:

Dilutions: working with live organisms

Complete:

MacConkey

Begin:

Characterization of the Unknown Organism
Macronutrient use – how organisms get energy to survive

Introduction: Hydrolysis and use of large extracellular materials

Inoculate:

- Polysaccharides: Starch plates
- Proteins: Skim milk plates and gelatin deeps
- Lipids: Rhodamine plates and Blood agar

Workshop topics to be determined by Instructor: Last 3 hours of lab day

Dilutions???

Presentation on scientific writing.

Computer lab 3060 and 3070 York Hall

Week 5

Lab 9: Tue/Wed May 2/3	Lab 10: Thu/Fri May 4/5
Complete: **Characterization of the Unknown Organism** **Macronutrient use – how organisms get energy to survive** • Polysaccharides: Starch plates • Proteins: Skim milk plates and gelatin deeps • Lipids: Rhodamine plates and Blood agar **Begin:** **Endospore test** **Inoculate:** • NSM with unknown only • IAs inoculate *B.sphaericus* and *L. plantarum* for reference **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** **Inoculate:** • Acid and gas from sugar fermentation • Methyl-Red and Voges-Proskauer **DISCUSSION/LEARNING TIME – 60-90 minutes** **HW3 Growth curve**	**Complete:** **Characterization of the Unknown Organism** **Endospore test** NSM –microscopy – unknown only Microscope clean up and check **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Acid and gas from sugar fermentation • Methyl-Red and Voges Proskauer **Begin:** **Inoculate fresh stocks of unknowns and control organisms:** • Unknowns (T-streak) • *Escherichia coli* • *Pseudomonas fluorescens* • *Enterococcus faecalis* • *Staphylococcus epidermidis* *IAs inoculate the following for the Nitrate Reduction test:* • *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 11 **Quiz 4**

Week 6

Lab 11: Tue/Wed May 9/10

Assign before class:

Before class: Kligler video part 1 & 2

Begin:

Characterization of the Unknown Organisms:

How energy is produced – aerobic vs. anaerobic breakdown of organic compounds

- Nitrate reduction
- Cytochrome C test
- Catalase test
- H_2S production – Kligler iron deep
- Oxygen requirements – thioglycolate tube

Motility

Inoculate:

- Plate and deep with unknowns and controls

How energy is produced – aerobic vs. anaerobic breakdown of organic compounds

Inoculate

- Nitrate reduction – nitrate broth

Inoculate fresh stocks for urease test in lab 12

1. *Enterobacter aerogenes*
2. *Proteus vulgaris*
3. Your unknown
4. ***IAs inoculate E. coli***

Inoculate 1 TSS or TSA of each **control** per aisle.

DISCUSSION/LEARNING TIME –
60-90 min
HW4 due: Complex dilution

Students come in on non lab day to check thioglycolate & Kligler iron deep

Lab 12 : Thu/Fri May 11/12

Assign before class: Regular reading

Complete Kligler video

Complete:

Characterization of the Unknown Organisms:

Motility

- Observe plates and deeps
- Observe wet mounts of controls and unknowns

How energy is produced – aerobic vs. anaerobic breakdown of organic compounds

- Nitrate reduction
- Cytochrome C test
- Catalase test
- H_2S production – Kligler iron deep
- Oxygen requirements – thioglycolate tube

Begin:

Special metabolic functions:

Inoculate:

- Indole production from tryptophan, catabolite repression
- Urease test
- Differential utilization of citrate by enterics

Complete all genus charts

DISCUSSION/LEARNING TIME – 60– 90 min.

Quiz 5

Student vote on Midterm 2 math day

Week 7

Lab 13: Tue/Wed May 16/17	Lab 14: Thu/Fri May 18/19
<u>Complete:</u> **Characterization of the Unknown Organisms:** **Special metabolic functions** • Indole production from tryptophan, catabolite repression • Urease test • Differential utilization of citrate by enterics **<u>Begin:</u>** Begin/inoculate any repeat tests **Extreme conditions** **Nitrogen fixation: Free-living - Anabaena** IAs inoculate BG11 and BG11-0 with *Anabaena* **IAs check all genus charts**	**Midterm 2 in lecture (Thursday)** **Dilution math in lab? – Students vote in advance** **<u>Complete:</u>** **Characterization of the Unknown Organisms:** Complete all repeated tests **<u>Begin & Complete:</u>** **Characterization of the Unknown Organisms:** Create elimination flow chart for identification of genus and species – computer lab 75 min genus flow chart 30 min species trial and eval 45 min BLAST and follow up **3060 and 3070 York Hall** **Extreme conditions: discussion** **Begin HW6 development**

Week 8

Lab 15: Tue/Wed May 23/24	Lab 16: Thu/Fri May 25/26
Midterm 2: dilution math in lab? – students vote in advance **Begin:** **Antibiotic Producers** Spread plates and grid out antibiotic producers and non-producers **Evaluation of antibiotics by the Kirby Bauer method** Spread plates with standards and test efficiency of antibiotics **DISCUSSION/LEARNING TIME – 60– 90 minutes: Begin HW6 development: Bring your own laptops**	**Assign before class:** **Transposon mutagenesis worksheet** **Begin:** **Nitrogen Fixation** 1. Free-living: *Anabaena*: check for heterocysts 2. Symbiotic*: Rhizobium:* Observe nodules **Yogurt - introduction** Review exp't with your group Formulate hypotheses Design an experiment IA must sign off on predictions **Complete:** **Antibiotic Producer** Evaluate Zones of Inhibition **Evaluation of Antibiotics by the Kirby Bauer Method** Measure ZOI, identify any resistant colonies **DISCUSSION/LEARNING TIME – 60– 90 minutes:** Quiz 6

Week 9

Lab 17: Tue/Wed May 30/31 **Assign before class:** **Transposon mutagenesis reading** _____ **Begin:Yogurt** Begin Yogurt: Inoculate control and experimental milk with starter culture and incubate under desired conditions Measure pH of uninoculated milk and sample yogurt Check pH, thickness at start (inoculated sample) Inoculate a fresh culture of *Staphylococcus* to use as a Gram+ control in staining, lab 18. **Yogurt:** Check pH, thickness at 3 hour time point Incubate overnight **Begin:** **Transposon mutagenesis:** Lab Period 1 Step 1: Set up conjugation of *E.coli* and *Citrobacter* or *Serratia* **DISCUSSION/LEARNING TIME/HW6 in-class work** HW5 due: Unknown ID	**Lab 18:** Thu/Fri **June 1/2** **Assign before class:** **Complete Transposon mutagenesis reading** _____ **Continue:** **Transposon mutagenesis:** Lab Period 2 • Step 2: Plate exconjugants for selection and counterselection **Complete:** **Yogurt:** • Measure pH. • Gram stain. Gram stain yogurt - commercial and/or experimental **Computer lab: HW6 development** **Quiz 7**

Week 10

Lab 19: Tue/Wed June 6/7 **Complete:** **Transposon mutagenesis:** Lab Period 3 • Count colonies and calculate transposition efficiency **Begin & Complete:** Lab clean up and check out Wrap and label lab coats for autoclaving **Computer lab: HW6 development** **HW6 development**	**Lab 20: Thu/Fri June 8/9** **Midterm 3** will be held in lab during regular lab hours

Finals Week

Tuesday, June 12/13, 1PM – HW6 due