Welcome to Microbiology Lab!
Outline For Today

• Enrollment
• Course Format
• Lab Schedule--next two weeks
• Format for pre-labs and lab notebook
• Background for this week:
  – Sterile Technique
  – Spread Plate/Enumeration and Streak Plating
  – Working safely with Biosafety level 2 organisms, part I
Enrollment issues

There are several open spaces, please sign up on the sheet up here for the Undergrad affairs office to assign those who get in.

Students with Disabilities: If you qualify for classroom accommodations because of a disability, please submit your Accommodation Authorization Letter from the Disability Resource Center (DRC) to me as soon as possible, preferably within the first week of this session. Contact DRC by phone at 831-459-2089 or by email at drc@ucsc.edu for more information."
Introduction: Instructors and TAs

• Instructor
  – Professor Karen Ottemann, Ph.D.

TAs:
  – Annah Rolig
  – Will Sause

Support Staff
  Amanda Coen
Everything is posted on the website

http://ic.ucsc.edu/~ottemann/metx119l/
## Course Overview (from the syllabus)

<table>
<thead>
<tr>
<th>Category</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Laboratory Reports (100 points each):</td>
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<td><strong>Total Points</strong></td>
<td><strong>656 points</strong></td>
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</tbody>
</table>
Every lab class meeting we have specific experiments to do:

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Activity</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Making Media</td>
<td>This week</td>
</tr>
<tr>
<td>2</td>
<td>Pipetting</td>
<td>This week</td>
</tr>
<tr>
<td>3</td>
<td>Isolating single colonies using streak and spread plates</td>
<td>Next week-monday</td>
</tr>
<tr>
<td>4</td>
<td>Bright field microscopy and Gram stain</td>
<td>Next week-monday</td>
</tr>
<tr>
<td>5</td>
<td>Phase contrast microscopy</td>
<td>Next week-Weds</td>
</tr>
<tr>
<td>6</td>
<td>Analyzing water samples for fecal coliforms and E. coli</td>
<td></td>
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<tr>
<td>7</td>
<td>Measuring bacterial growth using the spectrophotometer</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Isolating microorganisms from food</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Identifying bacterial strains using molecular techniques</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Determining antibiotic resistance properties</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Characterizing biochemical properties of bacterial strains</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group projects</td>
<td></td>
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</tbody>
</table>
For each lab your write a Pre-lab and keep a Lab Notebook

• 3 ring binder with dividers that separate each lab exercise.

• Print out and place a copy of the exercise instructions at the beginning of each section, and then follow that with your written description of what you did, using regular binder paper.

• Include the following:
  • A date on each page.
  • Number the pages as in 1-1, 1-2, for exercise 1 page 1 etc.
  • Continued next page…
Notebook II

• The first page of each section is the “prelab” information--
  – Title
  – Purpose
  – Simple list of methods
  – Example on next slide
• Next will come your methods.
  – More detailed than prelab.
  – Include exactly what you did
• Results and observations.
  – Includes graphs and figures
• Conclusion at the end of each exercise.
• Assignments: Sometimes the assignments are conclusions or graphs--you can hand these in and then put them back in the notebook
• Grading: The notebook will be graded on a 1-5 scale of poor (1, significant missing information) to excellent and complete (5). Your notebook will be inspected three times throughout the quarter, unannounced, so please keep it up to date!
Making LB Plates 9/28/10

**Purpose:** To learn how to make LB plates, including how to use the autoclave and sterile handling of media

**Methods:**
1. Weighing out materials (Tryptone, yeast extract and NaCl) and measuring water
2. Using the autoclave
3. Pouring plates
Assignments

• Each exercise has a set of assigned questions designed to teach scientific writing and data presentation. In some cases, the answers will be incorporated into your Lab Reports. Assignments are due generally 1 week following the end of that particular exercise. Exact due dates are listed on the web site in the Exercises/Due Dates page.
All exercises are on the Website:

<table>
<thead>
<tr>
<th>Date/Exercise</th>
<th>Assignments</th>
</tr>
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</table>
| **Bio119L Laboratory Manual** | Biosafety Assignment  
Monday 9/26/11  
Lab Safety and Course Introduction  
EXERCISE 1: Dilutions and Micropipettes  
Due: Biosafety Assignment  
Monday 10/3/11  
Enumeration by Spreading and Streaking Plates. Plates examined on 10/5  
EXERCISE 4: Bright Field Microscopy and Gram Staining  
Microscopy Readings (required):  
1) Basic Microscopy  
2) The microscope  
Due: Ex 1 Assignment (Pipetting)  
**Weds. 9/28/11**  
EXERCISE 2: Media Prep  
Due: Biosafety Assignment  
**Mon 10/10/11.**  
EXERCISE 6: Water quality analysis (multi-day)  
Group Projects: Form groups, work on proposal (proposal due 10/17). Great resource is *The Microbes Everywhere* web site.  
Due: Ex 4 Assignment (Bright Field Microscopy)  
**Mon 10/10/11.**  
EXERCISE 6: Water quality analysis (multi-day)  
Group Projects: Form groups, work on proposal (proposal due 10/17). Great resource is *The Microbes Everywhere* web site.  
Due: Ex 4 Assignment (Bright Field Microscopy)  
**Weds 10/12/11.**  
EXERCISE 7: Growth Curve  
Due: Ex 3 (Pipetting) and 5 (Phase Contrast) Assignment  
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Due: Ex 3 (Pipetting) and 5 (Phase Contrast) Assignment |
|                        | Ex. 1 assignment due 10/3/11  
Answers for self check of calculations  
Ex. 2 assignment due 10/5/11  
Sezonov Paper  
Ex. 4 due 10/10/11  
Ex. 3 due 10/12/11  
Ex 5 due 10/12/10  
Ex. 6 due 10/26/11  
MPN Table  
SC County Water Quality Page:  
EPA R323.1062 Microorganisms Water quality standards for total body contact (pdf)  
Assignment due 10/19/11  |
We will have two lab reports

• Lab reports will be written up in the format of a typical scientific research article. Each will contain these sections (1) Introduction and Hypothesis for the experiment; (2) Materials and Methods; (3) Results; (4) Discussion of results; (5) References.

• Specific guidelines will be given in advance of each report.
• The first lab report will be on the food lab, and will include a revision
Other components that you will be evaluated on:

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This week

Today

– Assign lab bench, hand out lab coats and sign out for drawers/microscopes
– Biosafety Assignment-due WEDS
– Dilutions and Micropipettes (Exercise 1)
  • No prelab
  • Dilutions portion is self-check
  • Pipetting will be demoed in the lab when you all have pipets in hand

Weds: Media Prep

• This needs to start as soon as your prelab is checked off.
• Put on Lab coats, locate materials and WEIGH!
Luria Bertani Broth is a type of undefined media

- Very rich, allows many microbes to grow
- Made of chopped up milk protein (tryptone), yeast and salt; not known exactly how much of a given ingredient is in it (undefined)
- Opposite is defined
We make the media from non-sterile ingredients, and then sterilize in the autoclave

- Temperature?
- Time?
We will use our plates to grow bacteria

• Single cell becomes many
• Restricted to one place
• http://www.youtube.com/watch?v=gEwzDydc1Wc&feature=related
Sterile Technique

• Basic Idea is that you keep sterile items closed and untouched by anything as much as possible
• Flame things like metal loops, or use sterilized items like tips or sticks for picking up bacteria
• Pass the tops of flasks through flames
• Close items quickly
Safety in the Microbiology lab
Lab Safety in Micro lab

The basic rule is to eliminate exposure to harmful pathogens or chemicals via

1) Proper personal protective equipment
2) Proper airflow and restrictions on the room
3) Proper waste management
Personal Protective Equipment

- You must have the right clothing to be in the lab:
  - Close-toed shoes and long pants.
  - You can keep lab clothes/shoes in the lab, just ask us.
  - You will not be allowed in without the proper shoes/pants.
- Tie back long hair or bangs
- Gloves are available and we will point out when they should be worn; you should always wash your hands after every exposure & at the end of the lab period
- Lab coats must be worn at all times
- Bring safety glasses or we have goggles
Nothing goes in your mouth

• Always using appropriate pipetting devices; mouth pipetting is forbidden
• Never eat or drink in the laboratory
  – There are tables outside the lab to leave your food/drinks. Of course, take off your gloves and wash your hands before you touch these things!
• Never apply cosmetics, handle contact lenses, or placing objects (fingers, pencils, etc.) in the mouth or on your face
We limit aerosols of potential pathogens: limits exposure

- Use wooden sticks instead of flamed metal loops
- High aerosol activities like blender in Biosafety cabinet
- Only enrolled students in the lab
No bacteria in the regular trash!

• All bacterial cultures, and things that have touched bacteria, are autoclaved
  – Petri dishes with media, cultures into special areas
• Special containers in the lab
• Some stuff can go in the regular trash like weigh boats, paper towels
• Glass always disposed of in special containers
  – Separated by bacterial exposure
At the end of the lab period

- Put away all the stuff you used in the proper places
- Clear your bench and wipe it down with disinfectant
- Clean up any common areas you used
- Wash your hands before you go off and eat food, touch your friends!
Other key safety stuff:

- Spills and accidents happen--so please don’t hesitate to report all to us.
- Report all spills and broken glassware to the instructor and we’ll give you instructions for clean up
- Know where to find the emergency equipment (eye wash stations, fire alarms, fire extinguishers, chemical safety showers, telephones, and emergency numbers)