You **MUST** attend lab during the first week of the term or you will automatically be dropped from MB 230.

**Instructors:**

Jesse J. Coutu  
coutuj@oregonstate.edu  
106A Dryden Hall
Label plates on the agar side, not the lid. Place plates agar-side up for most exercises.

Label culture tubes on the glass, not the lid.
<table>
<thead>
<tr>
<th>Week 1</th>
<th>Reading/Methods</th>
<th>Result</th>
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<td>v-vi</td>
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<tr>
<td><strong>Exercise 1:</strong> Use of the Microscope</td>
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<td><strong>Exercise 2:</strong> Microorganisms in the Air</td>
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<tr>
<td><strong>Exercise 2:</strong> Examine PCA plate</td>
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<tr>
<th>Week 3</th>
<th>Reading/Methods</th>
<th>Result</th>
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<td><strong>Exercise 6:</strong> Gram Stain</td>
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<td><strong>Exercise 7:</strong> <em>Rhizobium</em> &amp; Nitrogen Requirements</td>
<td>23-24</td>
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<td><strong>Exercise 4:</strong> Examine streak plate (Gram stain)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Exercise 5:</strong> Return and incubate RODAC and swab PCA plates</td>
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<th>Week 4</th>
<th>Reading/Methods</th>
<th>Result</th>
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<tr>
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<td>25-26</td>
<td></td>
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<td><strong>Exercise 9:</strong> Koch’s Postulates &amp; Crown Gall Disease (Gram stain)</td>
<td>27-28</td>
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<tr>
<td><strong>Exercise 10:</strong> Relation of Oxygen to Microbial Growth</td>
<td>29-30</td>
<td></td>
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<tr>
<td><strong>Exercise 5:</strong> Examine RODAC and PCA plates</td>
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<tr>
<th>Week 5</th>
<th>Reading/Methods</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td><strong>Exercise 11:</strong> Microbes of the Mouth (Gram stain)</td>
<td>31-32</td>
<td></td>
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<tr>
<td><strong>Exercise 12:</strong> Normal Throat Culture</td>
<td>33-34</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 8:</strong> Examine bacteriophage pour plates</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 9:</strong> Examine MGY plate &amp; Gram stain</td>
<td></td>
<td></td>
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<tr>
<td><strong>Exercise 10:</strong> Examine thioglycollate tubes</td>
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<thead>
<tr>
<th>Week 6</th>
<th>Reading/Methods</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td><strong>Exercise 13:</strong> Water and Coliforms</td>
<td>35-36</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 14:</strong> Conjugation &amp; Antibiotic Resistance</td>
<td>37-39</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 11:</strong> Examine sucrose plate and Gram stain</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 12:</strong> Examine blood agar plate and Gram stain</td>
<td></td>
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<thead>
<tr>
<th>Week 7</th>
<th>Reading/Methods</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td><strong>Exercise 15:</strong> <em>Staphylococcus aureus</em> in Potato Salad</td>
<td>40-42</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 16:</strong> Metabolism and Fermentation</td>
<td>43-44</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 13:</strong> Examine water bottles, set-up EMB plates</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 14:</strong> Examine antibiotic plates</td>
<td></td>
<td></td>
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<thead>
<tr>
<th>Week 8</th>
<th>Reading/Methods</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td><strong>Exercise 17:</strong> Cultured Dairy Products (Gram stains)</td>
<td>45-46</td>
<td></td>
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<tr>
<td><strong>Exercise 18:</strong> The Fungi</td>
<td>47-48</td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 13:</strong> Examine EMB plates</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 15:</strong> Set up potato salad dilutions; plate on MSA agar</td>
<td></td>
<td></td>
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<tr>
<td><strong>Exercise 16:</strong> Examine BCP sugar tubes</td>
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<tr>
<th>Week 9</th>
<th>Reading/Methods</th>
<th>Result</th>
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<tbody>
<tr>
<td><strong>Exercise 7:</strong> Examine clover plants for root nodules &amp; Gram stain</td>
<td></td>
<td></td>
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<tr>
<td><strong>Exercise 9:</strong> Examine carrots for crown gall &amp; Gram stain</td>
<td></td>
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<tr>
<td><strong>Exercise 15:</strong> Examine MSA plates &amp; Gram stain</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exercise 17:</strong> Examine tube of milk &amp; Gram stain</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Week 10</th>
<th>Reading/Methods</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>Turn in results pages; Take lab final.</td>
<td></td>
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</tbody>
</table>


Point Breakdown Per Week

These are the points you would miss if you don’t attend lab in the given week. There is no way to earn Results Points unless you attend the given lab. If you turn in your post-lab BEFORE the deadline by 1) sending it with a friend, 2) dropping it off to the instructor or 3) some other means so that your TA has it in hand BEFORE the deadline, you can still earn the post-lab points.

The Results packet is due Week 10, so if you fail to turn in your results packet you will not earn ANY of your results points.

<table>
<thead>
<tr>
<th>Week</th>
<th>Quiz Points</th>
<th>Results Points</th>
<th>Participation Points</th>
<th>Lab Final</th>
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<tbody>
<tr>
<td></td>
<td>Lab Safety Quiz</td>
<td>Pre-Lab Points</td>
<td>Post-Lab (PL) Points</td>
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</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3 (WK1 PL)</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>3 (WK2 PL)</td>
<td>1.5</td>
<td>1</td>
</tr>
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<td>4</td>
<td>4</td>
<td>4 (WK3 PL)</td>
<td>1.5</td>
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<tr>
<td>6</td>
<td>4</td>
<td>4 (WK5 PL)</td>
<td>2.5</td>
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<tr>
<td>7</td>
<td>4</td>
<td>4 (WK6 PL)</td>
<td>1.0</td>
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<tr>
<td>8</td>
<td>4</td>
<td>4 (WK7 PL)</td>
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<td>9</td>
<td>4</td>
<td>4 (WK8 PL)</td>
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<td>10</td>
<td>1</td>
<td>30</td>
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<td>10</td>
</tr>
<tr>
<td>Total Points</td>
<td>1</td>
<td>30</td>
<td>20</td>
<td>9</td>
</tr>
</tbody>
</table>

ii
MB 230 Laboratory Syllabus
Winter 2018

LAB SECTIONS: Wednesday 10:00 – 11:50 am, Friday 12:00 – 1:50 pm
Lab Rooms: Nash 304/316

LAB INSTRUCTORS: Jesse J. Coutu
coutuj@oregonstate.edu
106A Dryden Hall

GRADING POINTS: Lab-Safety Quiz ......................... 1
Pre-Lab Quizzes ............................................ 30
Post-Lab Questions ...................................... 30
In-Lab Results ............................................. 20
Lab Participation ......................................... 9
Lab Final ................................................... 10
TOTAL ....................................................... 100

TOTAL LAB POINTS = 25% of MB 230 grade
There is no curve for lab points

ATTENDANCE in lab is crucial! If more than one lab session is missed, you will receive a failing grade (F) for the entire MB 230 course. Except for extremely exceptional circumstances (as determined by the instructor), there is no way to make up a missed lab! All weeks count towards attendance.

Lab Participation Points: The participation points will be equally distributed for all the lab classes except the week 10 lab. These points will be given based on the signed attendance of the students on the attendance sheet. REMINDER: Students will not get the ‘Participation points’ for any make-up lab class.

Lab-safety Quiz: Lab-safety quiz will be available on Canvas before the first session of the scheduled lab and need to be submitted before the second lab session. All questions will be asked about the general safety of the lab that are discussed in this lab manual in the ‘Laboratory Safety’ section.

Pre-Lab Quizzes: Lab exercise quizzes will be available on Canvas before the scheduled lab sessions in which the exercises will be performed. The quizzes must be completed BEFORE coming to lab. Quizzes are available at the end of the previous lab session until the start of the next lab session.

In-Lab Results: Results will be recorded throughout the lab exercises and briefly checked by TAs in lab. The Results pages will then be turned in as a single packet on the final day of lab. Graded results will be available for pick-up from the instructor’s office during normal office hours.

Post-Lab Questions: A document containing all post-lab questions will be available on Blackboard and can be accessed throughout the term. Data for the post-labs will be collected during lab by your teaching assistant and posted to Blackboard. Post-lab questions should then be completed using the data during the week and turned in to your lab TA during the next lab session.

Lab Final: The lab final will be given on the last lab date of the session. The lab final will cover material from the all of the lab exercises.

Grade Appeals: Students have one week from the time that scores are given to contest a score.
Introduction to MB 230 Lab

The experiments in this manual have been selected to teach some of the basic techniques and organisms of microbiology. This course is appropriate for any student who wishes an introduction to the science of microbiology.

ATTENDANCE

ATTENDENCE in lab is crucial! If more than one lab session is missed, you will receive a failing grade (F) for the entire MB 230 course. Regular attendance is vital because all subsequent labs build upon prior weeks' work. There are no make-up labs.

Tardiness: Students are expected to be on time and be fully participatory for the scheduled lab time. Arriving at lab more than 10 minutes late two times will count as 1 absence. Arriving greater than 30 minutes late to lab will be considered an absence.

Attending a different MB 230 lab section: Attending other MB 230 lab sections is possible only with prior permission of your instructor and is subject to availability of open seats. Email request to your instructor as soon as you are aware of a time conflict with your scheduled lab time.

FORMAT

Each laboratory period begins with a lead-in lecture with explanations and demonstrations for that period's exercises. However, these lead-in lectures are not meant to replace your own preparations. Read the exercises before coming to lab!

On some exercises you will work independently and on others you will work in pairs or in small groups of students. See specific experiments for detailed descriptions of experimental formats.

Every time you use your microscope, you should write your name on the microscope sign-in sheet (inside microscope cabinet) and have your TA check your microscope before leaving lab.

Please ask questions at any time! The instructor and the teaching assistants will circulate and be available for help during the entire lab period.

LAB CLOTHING

Lab coats and safety goggles are not required. However, many reagents can produce permanent stains on your clothes and caution should be exercised to avoid this. To protect your belongings from such stains or microbial contamination, store your coat and book bag under your workbench. Only your MB 230 lab manual and lab supplies should be left on the workbench's surface during the laboratory period.

You will be working with open flame. Do not wear loose-fitting clothing, especially cloth bracelets or sleeves with dangling material. Long hair should be tied back.

Closed-toe shoes are REQUIRED; sandals and other such shoes are NOT allowed. Pants are recommended and shorts are advised against.

Double-check your belongings at the end of the lab period to make certain you have collected everything. The Microbiology Department will not be responsible for any items brought to lab.
1) **DO NOT** eat, drink or chew gum/tobacco. Open beverage and food containers must be left on the hallway shelf outside lab. Keep your hands out of your mouth, nose and eyes.

2) **KEEP YOUR WORKSPACE CLEAR** – Keep only the lab manual and necessary lab supplies on your bench top; everything else should be placed under the bench, keeping the aisles clear.

3) **WEAR APPROPRIATE CLOTHING** – a shirt and closed-toe shoes are REQUIRED. Flip-flops and halter tops are not recommended. If your attire is deemed inappropriate for lab work, you will be offered a lab coat for use or asked to leave the lab.

4) **NO UNAUTHORIZED VISITORS** in the lab. **NO ANIMALS** in lab.

5) **KNOW THE LOCATION** of the fire extinguisher in the hallway and the fire blanket & eye wash in the lab.

6) **CLEAN** desktop with **DISINFECTANT** at the beginning and end of class. Carefully wash hands with soap before leaving the lab.

7) **BUNSEN BURNERS** in the lab have almost invisible flames - turn them off completely when finished. Long hair must be tied back during lab to avoid contact with flame. Loose fitting clothing and clothing or jewelry with dangling material should not be worn.

8) **BROKEN GLASSWARE** - Call the instructor or TA to clean up the glass. **Do not dispose of any glassware in the regular garbage cans.**

9) **INJURY** - If you cut yourself in the lab, inform the instructor or TA so that the wound can be properly disinfected. If the injury needs professional assistance, you will be escorted to the Health Center or proper facility.

10) **SPILLAGE** – If you spill anything in lab, inform the instructor or TA so that they can clean up the spill. If culture is spilled on your clothing or belongings, they may require decontamination to assure your safety.

11) **INCUBATING** - Carefully label all materials to be incubated with your name/initials, seat number, and organism identification. Place materials to be incubated in the incubation tub at the front of your bench, unless otherwise directed.
   a. Label culture plates on **agar side** with your name/initials, seat #, and organism identification. Place plates in incubation tubs agar side up, unless otherwise stated.
   b. Label culture tubes on the **glass (not plastic caps)** with your name/initials, seat number, and organism identification. Place tubes in racks in incubation tub.
12) **DISCARDING CLASSROOM MATERIAL** - All materials used in this class that are contaminated with culture (tubes, plates, pipettes, etc.) must be autoclaved before cleaning or disposal; DO NOT throw these materials away in the metal cans on your bench tops nor in the normal garbage cans.

   a. **Contaminated ‘Soft Materials’**: Plastic Petri plates, transfer pipettes, swabs and other ‘soft’ materials go into an autoclave bag in a metal container (‘coffin’) on the discard table. Do not discard glass items in the autoclave bag!

   b. **Contaminated Non-Broken Glass**: Glass culture tubes go into wire baskets in metal containers (‘coffins’) at the discard table. Glass bottles go into metal containers (‘coffins’) at the discard table. Loosen screw caps before autoclaving.

   c. **Contaminated Broken Glass/Sharp Materials**: Used razor blades/pins go in the metal can for contaminated sharps.

   d. **Uncontaminated ‘Soft Materials’**: Paper towels used to clean the bench top with disinfectant or lens paper used to clean microscopes can be placed in the metal container on the lab bench. The metal container should be emptied into the main garbage cans at the end of each lab session.

   e. **Uncontaminated Non-Broken Glass**: Used slides should be cleaned with slide cleaner and returned to slide container.

   f. **Uncontaminated Broken Glass/Sharp Materials**: Used cover slips go in the cardboard box for glass waste.

13) **LEAVING THE LAB**

   a. Have TA check your microscope.
   b. Clear lab bench of all cultures, plates and other supplies.
   c. Empty the metal can containing uncontaminated ‘soft’ waste into a large garbage can.
   d. Clean desktop with disinfectant and wash hands with soap.
   e. Make sure you have all of your belongings before leaving lab.
**Exercise 1: Use of the Microscope**

**Objectives**
- Identify the component parts of a compound microscope.
- Correctly operate the microscope using various powers of magnification.
- Prepare and observe wet mounts and stained permanent slides.

**Reading**

In MB 230, you will use a compound microscope almost every lab period. A compound microscope uses two types of lenses - the ocular lenses and the objective lenses. These lenses work together to provide magnification or enlargement of objects. The total magnification is the product of the magnification of the objective lens and the magnification of the ocular lens. Magnification by each lens is indicated by markings on the lenses: 10X, 40X/45X, or 100X on the objectives; 10X on the oculars. Note: some microscopes have a 4X objective lens.

In addition to magnifying objects, microscopes enable the observer to distinguish structures that are separated by short distances. This function is called resolution. Resolution is more important than magnification: it is not always desirable to obtain the largest image possible, but it is necessary to obtain sharp detail. The ordinary light microscope with oil immersion (100X) objective can distinguish or resolve two points approximately 0.2 micrometers apart. This is defined as the limit of resolution. Another concept that is crucial to proper utilization of the microscope is its’ capacity to be parfocal. Parfocal refers to the microscope’s ability to remain in approximate focus when switching from one objective to another.

Of paramount importance to the microbiologist is the oil immersion (100X) objective of the microscope. All observations of bacteria in lab will require use of the oil immersion objective. To properly use this objective, one must actually place oil on the slide surface and immerse the objective in it by swinging it into place. The main effect of immersion oil is to collect aberrant light rays and allow them to enter the objective. Aberrant light rays are those which would otherwise be lost due to diffraction.

Specimens to be viewed with the compound microscope are mounted on glass slides. A stained permanent slide is prepared by fixing whole microorganisms or sections of organisms to the slide, staining the preparation, and covering it with a glass coverslip. The coverslip is attached to the slide by a transparent adhesive material (glue). Semi-permanent slides of bacteria are prepared by fixing and staining the preparation without gluing on a coverslip. Most of your microscope study in this course will involve semi-permanent slides that you will prepare yourself. Under normal circumstances the microbes in these preparations are dead.

An unstained wet mount is made by suspending microorganisms in a drop of water or other liquid and covering the drop with a coverslip. A wet mount dries after several minutes, so it cannot be used over a long period of time. In addition, because of the liquid between the slide and the coverslip, a wet mount can only be observed using the 10X and 40/45X objectives on the microscope. However, a wet mount enables the viewer to study living microorganisms.
Our microscopes are Leica and Leitz compound microscopes (see Figures 1.1 and 1.2 on the following pages). Each of the labeled parts in the figures should be identifiable on your own microscope. The basic parts and their functions are:

**Part Function**

<table>
<thead>
<tr>
<th>Part</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>Oculars (Eye pieces)</td>
<td>a series of lenses that usually magnify 10X</td>
</tr>
<tr>
<td>Width Adjustment</td>
<td>adjust for your eye span</td>
</tr>
<tr>
<td>Revolving Nosepiece</td>
<td>rotates to change from one objective to another</td>
</tr>
<tr>
<td>Objectives</td>
<td>usually three magnifications: 10X (low power), 40-45X (high dry power), and 100X (oil immersion); immersion oil used with this objective has a refractive index approximately the same as glass - light passes through without being bent or lost</td>
</tr>
<tr>
<td>Mechanical Stage Controls</td>
<td>moves the slide on two horizontal planes – backward &amp; forward and side to side</td>
</tr>
<tr>
<td>Movable Stage</td>
<td>raises and lowers during focusing</td>
</tr>
<tr>
<td>Diaphragm Lever</td>
<td>opens and closes the diaphragm to control the amount of light striking the object</td>
</tr>
<tr>
<td>Condenser</td>
<td>condenses light waves into a pencil shaped cone, thereby preventing the escape of light waves. Also controls the light intensity when raised or lowered</td>
</tr>
<tr>
<td>Condenser Knob</td>
<td>raises and lowers condenser</td>
</tr>
<tr>
<td>Light Control</td>
<td>turns light source on and off</td>
</tr>
<tr>
<td>Arm</td>
<td>supports upper half of the microscope</td>
</tr>
<tr>
<td>Coarse Adjustment</td>
<td>moves stage (or body tube) up and down rapidly for purposes of approximate focusing</td>
</tr>
<tr>
<td>Fine Adjustment</td>
<td>moves stage (or body tube) up and down very slowly for purposes of definitive focusing</td>
</tr>
</tbody>
</table>
FIGURE 1.1: The Leica Microscope

- Eyepieces
- Binocular Body
- Arm
- Diaphragm
- Infinity Corrected Objectives
- Coarse Focus Adjustment Knob
- Fine Focus Adjustment Knob
- Focusing Condenser Knob
- Rheostat Control Knob
- Condenser Alignment Screw
- Collector Lens
- Reversed Nosepiece
- Stage Finger Assembly
- Mechanical Stage
- Stage Adjustment Knobs
- Condenser
- Aperture Diaphragm control
- Base
FIGURE 1.2: The Leitz Microscope
GENERAL RULES FOR MICROSCOPE USE

The microscopes are valuable and should always be used with care!

PREPARING THE MICROSCOPE FOR USE

1. When you are ready to use your microscope, remove the microscope from cabinet by rolling shelf all the way out, then carefully lifting microscope onto the table. Bumping the oculars on the top of the cabinet can damage them.
2. Unwind the cord from around the oculars and plug it into a receptacle in the middle of the lab bench. Turn on the light source at the base of the microscope.

ADJUSTING LIGHT INTENSITY

Properly adjusting the amount of light for different magnifications will greatly enhance your ability to see details and make using the microscope much more comfortable.

1. Before examining a specimen make sure that your lamp is on and adjusted to full intensity. This ensures that all light waves reach your sample.
2. Find the control for your condenser lens height and adjust the condenser so that it is up close to the specimen.
3. For examining specimens with the low (10X) power objective, lower the light intensity by closing the iris diaphragm. This is the best way to control light level. When viewing a specimen using the high dry (40/45X) or oil immersion (100X) objective lenses, open the iris diaphragm completely to capture maximum light. After you have found an object to examine try reducing the light level using the iris diaphragm lever to see whether contrast is enhanced.

FOCUSING THE MICROSCOPE

Follow the detailed instructions on the following page.

PUTTING THE MICROSCOPE AWAY

1. After use, clean oil off the 100X objective by wiping with flat lens paper, followed by flat lens paper saturated with lens cleaner. Crumpled lens paper will scratch the lens. If oil is left on the objective, it will destroy the seal and the lens will need to be replaced (>300).
2. If oil is on the 40/45X lens, let the instructor or TA know so it can have special cleaning.
3. Remove dirt or oil from the stage, condensers or oculars if needed.
4. Store microscope with low power or blank objective in place and close to stage.
5. Wrap the cord around oculars neatly; replace in cabinet when shelf is completely rolled out.
6. Sign and date the sign-out sheet in the cabinet.
7. Have your TA check your microscope and initial sign-out sheet before leaving lab.
FOCUSING A MICROSCOPE

**Wet Mount**

1. Put slide on stage within movable slide holders.
2. Using mechanical stage controls, move the slide so that your specimen is directly over the light source.
3. Move the revolving nosepiece so that the **low power objective** (10X) is in place.
4. Using the coarse adjustment, move the 10X objective as close to the slide as possible.
5. Slowly turn coarse adjustment, raising the objective until specimen is in focus. Use the fine adjustment to obtain maximum clarity.
6. Without adjusting the focus, carefully change to the **high dry objective** (40/45X). Do not use this objective if it will touch the liquid on your slide.
7. Using fine adjustment, focus until the specimen is at maximum clarity.
8. Use the mechanical stage controls to move to optimal viewing areas. The fine adjustment may have to be changed as the field of view changes.
9. **DO NOT USE THE OIL IMMERSION LENS (100X) WITH A WET MOUNT.**

**Stained Permanent Slide**

1. Put slide on stage within movable slide holders.
2. Using mechanical stage controls, move the slide so that your specimen is directly over the light source.
3. Move the revolving nosepiece so that the **low power objective** (10X) is in place.
4. Using the coarse adjustment, move the 10X objective as close to the slide as possible.
5. Slowly turn coarse adjustment, raising the objective until specimen is in focus. Use the fine adjustment to obtain maximum clarity.
6. Without adjusting the focus, carefully change to the **high dry objective** (40/45X).
7. Using fine adjustment, focus until the specimen is at maximum clarity.
8. Without adjusting anything else, carefully rotate the nosepiece until the high dry objective is out of the way. Do not put the **oil immersion lens** (100X) in place yet.
9. Put a small drop of oil directly over the path of light on the specimen.
10. Carefully switch to the **oil immersion lens** (100X). The oil immersion lens should just barely touch the oil on the specimen. **ONCE THERE IS IMMERSION OIL ON YOUR SLIDE, YOU CANNOT GO BACK TO THE 40/45X OBJECTIVE! NOTIFY YOUR TA IF YOUR 40/45X OBJECTIVE GETS OIL ON IT.**
11. Using fine adjustment only, focus until the specimen is again at maximum clarity.
12. Use mechanical stage controls to move to optimal viewing areas. The fine adjustment may have to be changed as the field of view changes.
13. Always remember to clean the oil immersion lens with lens cleaner and lens paper when one is finished. **A MICROSCOPE SHOULD NEVER BE PUT AWAY WITH OIL ON IT.**
Wet mounts of pond water or hay infusions contain large numbers of unstained microorganisms. You may observe protozoa (amoebas, ciliates, or flagellates), various algae, some microscopic invertebrates, and the larger bacteria. Some of these microorganisms move very fast. Remember that only the 10X and 40/45X objectives should be used to observe wet mounts!

| TABLE 1.1: MICROORGANISMS FREQUENTLY OBSERVED IN HAY INFUSION OR POND WATER (Please note: drawings are NOT to scale!) |

<table>
<thead>
<tr>
<th>PROTOZOA (cells appear colorless, most are motile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Ciliomonas</em></td>
</tr>
<tr>
<td>2. <em>Paramecium</em></td>
</tr>
<tr>
<td>3. <em>Vorticella</em></td>
</tr>
<tr>
<td>4. <em>Euplotes</em></td>
</tr>
<tr>
<td>5. <em>Ciliophora</em></td>
</tr>
<tr>
<td>6. <em>Amoeba</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ALGAE (cells appear green or brown, may or may not be motile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Rhoplina</em></td>
</tr>
<tr>
<td>2. <em>Chlamydomonas</em></td>
</tr>
<tr>
<td>3. <em>Volvox</em></td>
</tr>
<tr>
<td>4. <em>Spirogyra</em></td>
</tr>
<tr>
<td>5. <em>Nostoc</em></td>
</tr>
<tr>
<td>6. <em>Stauronella</em></td>
</tr>
<tr>
<td>7. <em>Nostoc</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>COLONIAL BACTERIA: CYANOBACTERIA (cells appear blue-green or green, not motile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Aphanan</em></td>
</tr>
<tr>
<td>2. <em>Anacystis</em></td>
</tr>
<tr>
<td>3. <em>Arthrospira</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UNICELLULAR BACTERIA (cells appear transparent, may or may not be motile)</th>
</tr>
</thead>
</table>
EX. 1: Using a Microscope – Materials

Part A: Unstained Wet Mounts of Fluid Specimens
- pond water (containing algae and protozoa)
- hay infusion broth (containing protozoa and bacteria)
- microscope slides and cover slips

Part B: Stained Permanent Slides of Organisms
- eukaryote: Candida albicans (yeast)
- prokaryotes: Bacillus subtilis (large bacillus bacterium)
  Staphylococcus aureus (small coccus bacterium)

EX. 1: Using a Microscope - Methods

Part A: Unstained Wet Mounts of Fluid Specimens
1. Prepare a wet mount of pond water or hay infusion broth by placing a small drop of fluid on a clean microscope slide. Gently position the cover slip over the liquid.

2. Use directions on the previous pages for examining your unstained wet mounts. Remember to only use the 10X and/or 40/45X objectives on the microscope while observing your wet mount.

3. Draw observations in the RESULTS section. Protozoans are generally motile and clear in color. Algae, which may or may not be motile, tend to be greenish or brownish in color due to their pigments. The cyanobacteria also have pigments and will be green or blue-green in color. Most bacteria will be too small to see clearly at these magnifications.

4. When finished, rinse the slide with water and return to nearby slide holder. The coverslip may be disposed of in the cardboard box for broken glass.

Part B: Stained Permanent Slides of Organisms
1. The stained permanent slides of organisms have already been made for you. The cover slips have been glued on, so these slides can be used over and over again. Select a stained permanent slide of one of the three different microbes.

2. Use directions on the previous pages for examining your stained permanent slide. Remember to go all the way up to the 100X objective on the microscope while observing your stained slide. Draw observations in the RESULTS section.

3. After use, these slides should be cleaned and returned to their original box.
**Exercise 2: Microorganisms in the Air**

**Objectives**
- Acquire an awareness of the diversity of microorganisms in the air.
- Recognize the different types of microbial growth that occurs on a culture plate exposed to air.

**Reading**

No microorganisms grow in the atmosphere. Because air has no nutrients and little water, it is an inhospitable environment. When we speak of the microbial flora of air, we are referring to those organisms which are found temporarily suspended in air or carried about on dust particles or droplets.

A variety of techniques have been devised - some simple, some elaborate - for isolating and detecting microorganisms in the air. One of the simplest of these is the use of gravity (or settling) plates. Gravity plates are Petri plates filled with sterile media which are left open with the agar surface exposed. Microorganisms carried on dust or droplets simply "settle" on the agar surface and, following incubation, reproduce over and over to eventually form individual colonies.

The nutrient medium we will be using in our Petri dishes for this exercise is called **Plate Count Agar (or PCA)**. The media contains tryptone (digested meat protein), glucose (simple, easy to metabolize carbohydrate), and yeast extract (containing B vitamins and protein). These ingredients provide the basic nutrients required by many heterotrophic microorganisms. But not all microbes will grow on a PCA plate - microbes with complex nutritional needs and autotrophic microorganisms would not find the things they need to grow. The media is solidified using agar, a complex carbohydrate extracted from seaweed. Because of its complex structure and unusual origin most terrestrial microorganisms do not degrade it, unlike gelatin, an animal protein which is often degraded by common microbes. Agar reacts to heating much like gelatin. To dissolve agar in water it must be thoroughly heated. It will remain liquid while warm, solidifying at temperatures near 42°C (108°F). The nutrient medium has been **sterilized** by autoclaving at 121°C and 15 pounds/in² steam pressure. The combination of heat and pressure produced by an autoclave effectively kills nearly all microorganisms. The medium is then poured **aseptically** (without introducing unwanted organisms) into the plate and allowed to cool.

Agar plates are labeled on the agar side in case lids are accidentally switched at some point. Plates are typically incubated **agar side up** so that condensation that forms during incubation will not drip back down onto the colonies on the plate.

You will find that microorganisms are common in the air and on body surfaces. Preventing contamination of culture media by these organisms is difficult. The microbiologist must work carefully using a special set of procedures called **aseptic technique** to prevent organisms from
inadvertently entering sterile media. You will learn aseptic technique in a later laboratory exercise.

**EX 2: Microorganisms in the Air - Materials per Student**

1 sterile plate count agar (PCA) plate

**EX 2: MICROORGANISMS IN THE AIR: Methods**

1. Label the plate on the *agar side* with your name and seat number.

2. Remove the Petri plate lid and expose the agar surface to the air until the end of lab. You may gently touch the agar surface with one finger if you would like. Note the delicate texture of the agar. In later lab exercises you will be depositing organisms across this rather fragile surface.

3. At the end of lab, replace the lid, turn the plate over so that the agar side is up, and place the plate in the correct box for incubation.

4. Next lab, examine your plate and note the types of microbial colonies that have grown on the agar surface. Contrast the colonial growth on your plate with those around you. If you touched the plate, note any differences in the density of the colonies on that part of the plate with other areas of the plate. Record observations in the **RESULTS** section.

<table>
<thead>
<tr>
<th>Table 2.1: Characteristics Used for Colony Evaluation on Agar Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Pigmentation:</strong></td>
</tr>
<tr>
<td><strong>Form:</strong></td>
</tr>
<tr>
<td>shape of the colony</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Margin:</strong></td>
</tr>
<tr>
<td>appearance of colony outer edge</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Elevation:</strong></td>
</tr>
<tr>
<td>degree to which colony growth is raised on agar surface</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Exercise 3: Morphology of Bacteria – The Simple Stain

Objectives
• Prepare bacterial smears and apply the simple stain technique.
• Distinguish among basic bacterial shapes (morphologies) and cell arrangements.

Reading
When bacteria are stained using a single dye, it is called a **simple stain**. The dye enhances the contrast between the cell and its surroundings, showing us the **morphology** or shape of the cell. **Dyes** are generally salts in which one of the charged particles or **ions** is colored. A salt is a compound composed of a positively charged ion and a negatively charged ion. The simple dye methylene blue is actually the salt methylene blue chloride, which dissociates in water to produce:

\[
\text{MbCl} \rightarrow \text{methylene blue} + \text{chloride}
\]

The color of the stain is in the positively charged methylene blue ion. Bacterial cells have a slight **negative charge** when the pH of their surroundings is near neutrality (which it generally is). The negatively charged bacterial cell combines with the positively charged methylene blue ion, with the result that the cell is stained. It is the **difference in charge** that produces an affinity between the dye and the bacterial cell.

The three general morphologies (shapes) of bacteria are:

- **coccus**
- **bacillus**
- **spiral**

Apart from these differences in cellular shape, definite patterns in cellular aggregations or arrangements are known to exist among different bacteria. In the case of spherical or **coccus** forms, two important patterns include chains of four or more organisms (**streptococci**) and irregular groups of microorganisms resembling grape clusters (**staphylococci**).

**Bacilli** (**sing.**, bacillus) are also referred to as rod-shaped bacteria.

Three groups of **spiral-shaped** bacteria are known. The **spirochetes** consist of flexible, waving forms, with several coils. The second group, the **spirilla**, is a group of rigid bacteria possessing one or several curves. The third group, the **vibrios**, consists of short, curved bacteria.

Variations in the general shapes and sizes of bacteria are frequently seen, and can be explained in terms of environmental factors. **Pleomorphism** is the term used to denote these variations.
FIGURE 3.1: Steps to Prepare a Bacterial Smear

The last step kills the bacteria and fixes the cells to the slide. Do not overheat!
EX 3: Morphology of Bacteria – Materials

- PCA plate from Exercise 2 (per student)
- Broth cultures: *Escherichia coli*
  - *Staphylococcus epidermidis*
- Prepared slide of *Spirillum*
- Bottle of methylene blue stain

EX 3: Morphology of Bacteria – Methods

Making a Bacterial Smear

It is absolutely imperative that the slide has been cleaned thoroughly or bacteria will not adhere to the slide and will be washed off during the staining process.

Cleaning a Slide:
- Thoroughly wash with slide cleaner.
- Rinse with water and blot dry.
- Lightly flame with Bunsen burner.

Avoid touching the cleaned slide surface with your fingers, or oils will be re-deposited on the slide. Instead, hold the clean slide by the edges.

Making a Smear:
1. A small amount of bacterial growth is placed in the slide.
2. Spread the organisms over the surface of the clean glass slide.
3. The thin film, or bacterial smear, is air-dried on the slide.
4. Pass the slide briefly through the flame of a Bunsen burner once or twice.

The last step kills the bacteria and fixes the bacterial cells to the slide. Do not heat the smear before it is completely air dried and do not overheat the smear or the bacteria will explode and lose their shape.

When making a smear from a solid medium, you should place a small drop of distilled water on the slide first. The less water you can use, the faster the smear will dry.

Methylene Blue Simple Stain

1. Apply enough methylene blue stain to cover the fixed smear. Let it sit for one (1) minute.
2. Pour off the stain. Rinse slide with slowly running water.
3. Blot the slide dry by placing it between two paper towels and pressing gently. Do not rub slide.
EX 3: Morphology of Bacteria – Microscopic Examination

1. Make a smear from one colony of the PCA plate from exercise 2. Use a small drop of distilled water to make this smear. Stain the smear with methylene blue. Examine the slide with your microscope.

2. Make a smear from the *Staphylococcus epidermidis* broth culture. Stain the smear with methylene blue. Examine the slide with your microscope.

3. Make a smear from the *Escherichia coli* broth culture. Stain the smear with methylene blue. Examine the slide with your microscope.

4. Examine the slides with your microscope.
   a. Start with the low power (10X) objective. Find the microscope focal plane with color, bring the colored object(s) into focus and center in microscope field of view.
   b. Turn the high dry objective (40/45X) objective into place over the specimen. Refocus and re-center the colored object(s).
   c. Move the high dry objective out of the way, add a drop of immersion oil on top of the slide and move the immersion oil (100X) objective into place above the specimen. Refocus the objects and record results.
   d. Clean the immersion oil off of the microscope before beginning the process over with another prepared slide.
**Exercise 4: Pure Culture Technique – Preparing a Streak Plate**

**Objectives**
- Employ the streak plate technique to produce individual colonies on an agar plate.
- Practice aseptic technique.

**Reading**
In order to study microorganisms and observe their characteristics it is first necessary to obtain them in pure culture (a pure culture is defined as a culture containing only one species of organism). This is very important because it is impossible to study the characteristics of a microorganism when contaminants (unwanted organisms) are present.

One of the most common methods employed in the laboratory to obtain a pure culture is the preparation of a **streak plate**. To properly streak a plate for isolation you must spread out the organism(s) by means of the inoculating loop until single colonies result. Each single colony consists of a cluster of cells that originate by cell division from a single bacterial cell. **Thus each isolated colony represents a pure culture of bacteria.** To do this, you must first learn **aseptic technique**. This is a special set of procedures designed to prevent contamination. This means that you transfer only the microorganism of interest and do not contaminate them with other microorganisms from the surrounding environment.

**Rules for Aseptic Technique**
- Bacteria are found everywhere, use common sense to avoid contamination.
- You must not allow any part of yourself or any other non-sterile object to touch the growth media.
- Do not remove the lid from your Petri dish completely, instead lift it and hold it above the dish to protect the media from dust.
- Do not put test tube caps or Petri dish lids down on the counter.
- Sterilize your loop or inoculating needle by heating it until it glows. Do this before and after each transfer.

![Figure 4.1 Separation of bacteria using the streak plate](image)

**First Streak** – Pick up bacteria using a sterilized loop; streak bacteria on the top third of your plate.

**Second Streak** – Flame the loop. Touch the hot loop to the agar to cool before going across the previous streak once, then streaking the second section.

**Third Streak** – Flame the loop. Cool and streak from section 2 to 3, only going once across your second streak.
Inoculate new tube or plate using aseptic technique with new tube/plate.

Figure 4.2
EX 4: Streak Plating – Materials

broth culture: mixed culture containing both Micrococcus luteus and Escherichia coli
inoculating loop
1 trypticase soy agar (TSA) plate (per student)

EX 4: Streak Plating - Methods

1. Follow the procedure in Figure 4.2 to aseptically pick up a loopful of bacterial broth culture. Follow the procedure in Figure 4.1 to spread the loopful of culture onto the agar surface, covering ≈ ⅓ of the surface in a back-and-forth fashion. Keep your loop on the agar surface and don't gouge the agar. In this area you should get confluent growth after incubation.

2. Reflame your loop, killing all of the bacteria on its surface. Touch the hot loop to the agar surface in order to cool it down before going across your first streak once, and then proceeding to streak the second section of the agar. In section two, you should get moderate growth after incubation.

3. Reflame your loop, again killing all of the bacteria on its surface. Touch the hot loop to the agar surface in order to cool it down before going across the area of your second streak once and then proceeding to streak the third section of the agar. In section three, you should get discrete colonies after incubation, because the bacteria will have been diluted out sufficiently from the original culture.

4. Label the agar-side of the plate with your initials/seat # and ‘Mix’ to indicate the bacterial culture used. Incubate plate agar-side up in the appropriate incubation box until next lab period.

5. Next lab, observe the plates and answer questions in RESULTS section.
Exercise 5: Environmental Sampling – Microbial Examination of Surfaces

Objectives
• Apply Public Health sanitary levels to microbial samples gathered from the environment.
• Recognize the correlation between the number of microbial colonies obtained from an object with the degree of sanitation.

Reading
The contamination of food service utensils, equipment, and food preparation counter tops is of considerable interest to individuals in a number of health-related occupations. Such occupations include people who are health inspectors, food processors, and hospital epidemiologists. These people need to be able to judge accurately the degree of cleanliness of many objects. In order to accomplish this, several techniques have been devised.

The most simple and rapid procedure is the use of the **RODAC plate** to obtain a direct environmental sample. The RODAC plate is a special plate in which the agar has been slightly overfilled. When the cover is removed it can be seen that the agar protrudes slightly above the lip of the plate, giving a convex surface. In use, this agar surface is pressed lightly on the surface being sampled, the lid is replaced, and the plate is incubated. Organisms on the surface sampled adhere to the agar and grow as discrete colonies which may then be counted. Thus, an approximate idea as to the degree of sanitization can be obtained. RODAC stands for **Replicate Organism Detection and Counting**. The limitation of the RODAC plate is that it can only be used successfully on flat, impervious surfaces which are free of crevices. Additionally, not all microbes can utilize the nutrient agar in the RODAC plate as food.

A second method which has the distinct advantage of being useful on irregular surfaces is the **moist swab (or utensil swab) technique**. Here a sterile swab is moistened with a special phosphate buffered solution and is then used to wipe a defined area of eating utensils such as glasses, forks, spoons, etc. The swab is then returned to the vial containing the diluting fluid, tightly capped, and transported back to the laboratory. The diluent keeps the organisms in an osmotically correct environment so that they remain alive during transport back to the laboratory. Once at the lab, 0.25 ml of the fluid is plated out on appropriate medium. Following incubation, the plates are observed and the colonies counted.
EX. 5: Environmental Sampling – Materials per Student

1 paper bag to be taken home containing:
   1 RODAC plate
   1 tube of buffer containing sampling swab
   1.0 ml sterile transfer pipette (used in lab)
   1 PCA plate (used in lab)
   hockey stick and flaming alcohol (used in lab)

EX. 5: Environmental Sampling – Methods

TO BE DONE AT HOME: Inoculate the night before or morning of your lab.

RODAC Plate: Use on any flat surface (e.g. cutting board or kitchen countertop).

1. Remove the lid, press exposed agar firmly but gently onto surface being sampled using a slight rocking motion. Replace lid and re-tape to prevent the lid from falling off. Label the plate with surface sampled. Bring the RODAC plate back to class the following lab period.

Sample Swab: Use on four spots of any irregular surfaces (e.g. four utensils or four spots on a keyboard).

1. Bend the red snap valve until you hear the valve break. Squeeze the bulb of the swab to transfer all of the liquid to the tube end with the swab. Twist and pull apart the bulb end of the swab from the tube end of the swab.

2. Swab any irregular surface, by rolling the moist swab back and forth 3 times over the surface. After this is done, the swab is immersed back into the buffer, swirled, and pressed out against the side as before.

3. Using the same swab procedure as before, repeat on another clean utensil/cup until a series of 4 similar utensils/cups (or surfaces) have been swabbed. When the fourth surface has been swabbed, replace swab in sampling tube for transport to lab. Be sure to **tightly cap** the sampling tube.

4. Label the sampling tube with the type of surfaces swabbed so you don’t forget. Bring the sampling tube back to class the following lab period. The instructions for plating the sample are listed on the next page.
BACK IN THE LAB

RODAC Plate:

1. Label the RODAC plate with your initials/seat # and the site sampled before placing in the correct incubation tub. Plates will be incubated for 48 hrs at 37°C.

Sample Swab:

1. Before beginning, label your PCA (plate count agar) plate on the agar side with your initials/seat # and surface sampled.

2. Remove a 0.25 ml aliquot of the buffer using a 1.0 ml sterile transfer pipette. Place the aliquot onto the agar surface.

3. Sterilize a glass "hockey-stick" by dipping it into flaming ethanol and lighting the ethanol on fire. DO NOT HOLD THE HOCKEY STICK IN THE FLAME. Allow the ethanol to completely burn off and then use the hockey stick to spread the liquid evenly across the agar surface. Rotate the plate to allow for even distribution. Place the plate in the correct incubation tube. If the agar surface is still quite wet, do NOT place agar side up. Plates will be incubated at 37°C for 48 hrs.

Interpretation of Results:

After colonies have grown interpret the RODAC plate and PCA plate as follows:

1. Count the colonies on your plates. **If colony count exceeds 100, sanitation is definitely not acceptable.**

2. Describe the most common colony type on each plate (color, shape, size).

3. Select a single colony from your RODAC plate; make a smear and Gram stain, following the instructions from Exercise 6. Be sure to place a small drop of water on your slide to mix in with the selected colony.

4. Record your results in **RESULTS** section.
Exercise 6: The Gram Stain

Objectives
• Practice Gram staining bacterial smears.
• Acquire a general understanding of the theoretical explanation for differing Gram stain reactions.

Reading
Simple stains do not tell us much about the cell except for morphology (shape). If we wish to learn more about the cell, such as whether it contains a spore or not, its cell wall type, or whether it possesses a capsule, we must use a differential stain. Differential stains normally employ several dyes. The Gram stain is a differential stain; it allows us to differentiate bacteria by their cell wall structure. Gram positive cells have a thick layer of peptidoglycan, a sugar-amino acid complex. Gram negative bacteria have a thin layer of peptidoglycan and an outer lipid membrane. A Gram stain is often the first step in the differentiation and identification of bacterial species.

The Gram stain requires the use of four reagents. The first dye or primary stain added to the smear is crystal violet, which is followed by an iodine solution. The iodine is called the mordant (a specialized term used in dyeing), which combines with the crystal violet to form an insoluble colored compound in the microbes being stained. This insoluble precipitate is called the crystal violet-iodine complex. After decolorizing, usually with acetone-alcohol, safranin dye is applied to the smear as a counterstain.

Organisms which resist decolorizing and retain the crystal violet-iodine complex appear purple or blue and are called Gram positive. Conversely, those cells that decolorize or give up the crystal violet-iodine complex will accept the safranin counterstain and appear red or pink. These are the Gram negative organisms.

You should remember that the differentiation of the Gram reaction is not an absolute, all-or-none phenomenon. It is based on the rate at which the cells release the crystal violet-iodine complex to the decolorizer. Even Gram positive organisms can show a Gram negative reaction if decolorized too much. A number of other factors can result in variable Gram reactions (where some cells appear Gram positive and others appear Gram negative), such as the following:

1. Improper heat fixing of the smear. If a smear is heated too much, the cell walls can rupture, causing G+ cells to release the crystal violet-iodine complex and accept the counterstain.
2. Cell density of smear. An extremely thick smear may not decolorize as rapidly as one of ordinary density.
3. Length and thoroughness of washing after crystal violet.
4. The amount of decolorizer applied. Eventually Gram+ cells will decolorize.
5. Age of bacterial culture. Gram reactions are only reliable for cultures 24 hours old or less. Cultures older than 24 hours have increased cell wall permeability and thus may convert from an original Gram positive reaction to a Gram negative reaction.
EX. 6: Gram Staining - Materials

Gram stain reagents: crystal violet, iodine, acetone-alcohol and safranin broth cultures: *Micrococcus luteus* and *Escherichia coli* trypticase soy agar (TSA) plate from exercise 4

EX. 6: Gram Staining - Methods

Gram Stain: A Differential Stain

1. Apply enough crystal violet (primary stain) to cover the fixed smear. Let it sit for one minute.
2. Pour off the stain. Rinse slide with slowly running water.
3. Apply enough iodine (mordant) to cover the fixed smear. Let it sit for one minute.
4. Pour off the iodine. Rinse slide with slowly running water.
5. Tilt slide over paper towel. Drip acetone-alcohol (decolorizer) down top of slide, so that it runs down the slide over the smear and onto the paper towel. Continue dripping decolorizer until it runs clear off the slide (approximately 4-5 seconds).
6. Immediately rinse slide with slowly running water.
7. Apply enough safranin (secondary counter stain) to cover the fixed smear. Let it sit for one minute.
8. Pour off the safranin. Rinse slide with slowly running water.
9. Blot the slide dry by placing it between two paper towels and pressing gently. Do not rub slide.

Microscopic Examination

1. Prepare a smear from each of the above bacterial cultures and from a colony from your TSA plate. (Ex. 3 has specific instructions on how to make smears.) Air-dry and heat-fix the smears before beginning the staining procedure.
2. Stain the smear using the Gram stain (detailed above)
3. Examine your slide with the microscope, starting out with the low power (10X) objective and working your way up to the oil immersion lens (100X objective) of the microscope. Be sure to get everything in focus before moving on to the next objective.
4. Under the 100x objective, note whether the bacteria are Gram positive or negative, based on their color. Also note the morphology (shape) of the bacteria under the microscope. Record observations in RESULTS section.

<table>
<thead>
<tr>
<th>Step</th>
<th>Flood w/</th>
<th>Time</th>
<th>Gram+</th>
<th>Gram-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. primary</td>
<td>crystal violet</td>
<td>1 minute</td>
<td>Rinse w/water</td>
<td>Purple</td>
</tr>
<tr>
<td>2. mordant</td>
<td>iodine</td>
<td>1 minute</td>
<td>Rinse w/water</td>
<td>Purple</td>
</tr>
<tr>
<td>3. decolorize</td>
<td>acetone-alcohol</td>
<td>≈5-8 seconds</td>
<td>Rinse w/water</td>
<td>Purple</td>
</tr>
<tr>
<td>4. counterstain</td>
<td>safranin</td>
<td>1 minute</td>
<td>Rinse. Blot dry.</td>
<td>Purple</td>
</tr>
</tbody>
</table>
Exercise 7: *Rhizobium* and Nitrogen Requirements for Legumes

**Objectives**
- Acquire an understanding of the importance of *Rhizobium* nitrogen fixation for the nitrogen requirements of leguminous plants
- Locate *Rhizobium* bacteroids in root nodules

**Results**

Plants, like all organisms, need nitrogen to produce proteins, nucleic acids and other polymers which are crucial for their cell structures and metabolism. Nitrogen gas (N\(_2\)) constitutes 80% of the earth's atmosphere, but plants cannot use nitrogen in this form. Nitrogen-fixing prokaryotes however can turn N\(_2\) into organic nitrogen which can then be used by plants. Some plants such as legumes (peas, beans, clover and alfalfa) nurture a mutualistic symbiosis with nitrogen-fixing bacilli from the genus *Rhizobium*. The legume produces enlarged sections of root tissue called root nodules where *Rhizobium* bacteria develop into a pleomorphic form known as bacteroids and fix nitrogen into organic nitrogen for use by the legume. Root nodules are variable in size, 1-10 mm. *Rhizobium* bacteria actively induce root nodule formation by invading and infecting root cells. The bacterium receives energy, usually in the form of carbohydrates from the host plant. Because of this symbiotic relationship, peas, beans, and clover do not need nitrogen-containing fertilizers to grow well and can actually enrich the soil with fixed forms of nitrogen which can be used by other plants. Rotating legumes with other crops is a popular agricultural strategy which allows farmers to minimize the use of nitrogen fertilizer. Without their microsymbionts, legumes require nitrogen in a fixed form and do not grow well in nitrogen poor soil. The use of nitrogen rich fertilizers may discourage root nodule formation.
EX. 7: Nitrogen Usage – Materials per Pair

Lab 1

*Rhizobium* inoculum: *Rhizobium leguminosarum* biovar *viceae* and *Bradyrhizobium* sp. biovar *phaseoli*

- 1 container of 6-8 clover seeds
- 1 sterile pot with potting soil
- 1 tube of 5ml sterile water
- 2 plant markers

Lab 2

- butcher paper for uprooted plant

EX. 7: Nitrogen Usage – Methods

Lab 1: You will receive ONE of the following seed containers...

Control Seeds: Add 1 tube of sterile water to container holding clover seeds. Swirl gently. Plant seeds in pot containing sterile soil, approximately 1 cm below soil surface. Use several seeds per pot. Label marker stick with CONTROL.

Inoculated Seeds: Add 1 tube of sterile water to container holding clover seeds. Pour in *inoculum* (fine black powder) and swirl gently to coat. Plant the mixture in a pot containing sterile soil approximately 1 cm below soil surface. Label marker stick with INOCULATED.

Pots will be watered and maintained at room temperature without the application of fertilizer.

Lab 2

1. Observe the health/vigor, and color of the control versus inoculated plants. Measure the height of each plant from where the plant comes out of the soil to the topmost leaves.

2. Carefully uproot plants. Shake off excess soil, gently rinse roots with water and look for the presence of small (1-10 mm) grayish-white nodules attached to the roots.

3. If root nodules are present: remove one nodule with fingers, brush off dirt and place it on a clean slide. Add a small drop of distilled water. Use another clean slide to crush the nodule and spread it around on the first slide. Gram stain and observe using 100X objective for the bacteroid form of *Rhizobium*.

4. Record observations in the RESULTS section.

5. When finished discard plants and soil in the trashcan. Do not throw away pots! Stack pots at the discard center by the autoclave bag.
Exercise 8: Isolation of Bacteriophages

Objectives
- Memorize steps in the replication of bacteriophage in bacteria.
- Acquire an understanding of how plaques are produced by the plaque method.
- Practice calculating the concentration of PFU/ml (plaque-forming units per milliliter) using plaque counts and dilution information.

Reading
Viruses capable of infecting bacteria are called bacteriophages, or phages. To start an infection, the phage attaches onto the surface of a bacterial cell by means of its tail fibers and base plate. The phage injects its DNA or RNA into the bacterial cell during entry. During penetration, the tail sheath contracts, driving its core through the bacterial cell wall and thus pushing viral DNA into the bacterium. In the synthesis stage, the bacterial cell is forced to make viral components, such as viral nucleic acid and proteins. These viral parts are put together during assembly, and the completed viruses are finally freed from the bacterial cell during the release stage.

Bacteriophages can infect bacteria grown in liquid (broth) or solid cultures. The use of solid media makes visualization of the phage possible by the plaque method. In the plaque method, host bacteria cover the surface of an agar plate to produce a confluent "lawn" of growth. Bacteriophages are then added in a discrete spot on this bacterial lawn. At this point, each bacteriophage that infects a bacterial cell will multiply until the bacterial cell is burst open or lysed. The new viruses that are thus released will infect adjacent bacterial cells and similarly produce more viruses. Eventually, many bacteria in one area will be destroyed, leaving a clear area or plaque within the confluent lawn of uninfected bacterial cells. The number of plaques on each plate is counted and this information, along with dilution information and volume, is used to determine the original concentration of virus as Plaque Forming Units (PFU) per milliliter (PFU/ml). The most accurate estimate is achieved using plate(s) with a plaque count in the 25-250 range. Less than 25 plaques on a plate could be due to bubbles or bits of solidified agar, while more than 250 plaques on a single plate are difficult to differentiate.

In this exercise, Lactococcus lactis, a bacterium used in the acidification and clotting of milk during cheese production, will be used as a host for bacteriophage c2. You will allow the virus to attach to the host cell and then add them to molten agar. The agar will be layered on top of an NA (nutrient agar) plate where virus replication and host cell lysis can occur. This layering is referred to as a pour plate.
EX. 8: Bacteriophages – Materials per Pair

0.1 ml bacteriophage c2
0.2 ml Lactococcus lactis broth culture

1 sterile long plastic transfer pipette
1 tube of 5 ml soft agar at 48°C
1 trypticase soy agar (TSA) plate

EX. 8: Bacteriophages – Methods

Lab 1

1. Write your names/seat numbers and the dilution factor (10^4 to 10^9, recorded on your virus tube) on the agar side of your TSA plate.

2. Using the plastic transfer pipette, remove the L. lactis from their tube and put them into the tube containing the bacteriophage c2. Gently swirl mixture.

3. Allow mixture to sit for 5 minutes on your lab bench.

4. After the 5 minutes are up, remove one tube of soft agar from the 48°C water bath.
   a. Dry the bottom of the tube off so that the water from the water bath does not contaminate your culture and work quickly for the remaining steps.
   b. Add the mixture of virus and bacteria to the tube of soft agar
   c. Swirl the tube and pour it out onto the TSA plate. The agar will set up quickly, so don’t waste time!
   d. Gently swirl the plate so that the agar will spread evenly across the surface.

5. Wait at least 10 minutes for the agar to become completely solid and place the plate in the box to be incubated. Plates will be incubated at 30°C for 4-12 hours.

Lab 2

1. Examine an entire set of dilution plates (10^4 to 10^9), looking for plaques formed in the L. lactis lawn. Completely cloudy plates have no plaques (solid lawn of bacteria not destroyed by bacteriophage); clear plates indicate that the bacterial lawn has been completely destroyed by bacteriophage. Count the number of plaques on each plate, recording clear plates or mostly clear plates as TNTC (too numerous to count).

2. Record your plaque counts in the RESULTS section. Use the results to determine the concentration of virus in the original suspension as Plaque Forming Units per ml (PFU/ml). The most accurate count is achieved using any plate with a plaque count in the 25-250 range.
Exercise 9: Koch’s Postulates – Crown Gall Disease in Plants

Objectives
• Isolate *Agrobacterium tumefaciens* and observe its morphology.
• Apply Koch’s Postulates to prove that *A. tumefaciens* causes crown gall disease in plants.

Reading

Crown gall disease is caused by the soil bacterium, *Agrobacterium tumefaciens*. Crown gall may occur in trees, roses, tomato plants, sunflowers, and other broad-leaf plants. The disease process starts when *A. tumefaciens* bacteria enter wounded plant surfaces, often the roots. *A. tumefaciens* transfers some of its DNA into the plant cell DNA, using a plasmid known as the Ti (tumor-inducing) plasmid, which contains genes for proteins that stimulate bacterial reproduction. The bacterial DNA also transforms host plant cells into tumors cells, eventually causing the formation of galls, a tumor-like growth. Galls are most common on roots and shoots. If they are at the crown, where the stem comes out of the soil, or on the main roots, plants grow poorly or die. Once the disease is established, the tumor continues to grow, even if viable *A. tumefaciens* bacteria are eliminated.

We will use this plant disease to illustrate Koch's postulates. Koch’s postulates are used to establish that a particular disease is caused by a particular microorganism. It works well for many bacterial diseases and was instrumental in demonstrating that microbes can cause disease. This concept is so widely accepted today that it is difficult to appreciate the confusion induced by contaminating microorganisms, poorly designed experiments, and plain bad luck, which had beset Koch’s fellow scientists in the 1870’s. The German country doctor published a paper containing the following proof. The particular disease he was investigating was anthrax.

Koch’s Postulates:
1. Observe the microorganism in a host with disease symptoms.
2. The microorganism must be isolated from the diseased host and cultured in pure form.
3. The pure culture of the microorganism, when inoculated into a healthy organism, must cause the same disease symptoms.
4. The microorganism must be re-isolated from the artificially-infected host and shown to be the same as the original isolate.
**EX. 9: Koch’s Postulates – Materials per Pair**

First lab:  
diseased carrots (carrot slice demo and piece of tumor tissue)  
buffer solution containing diced tumor  
MGY plate

Second lab:  
healthy carrot slice  
Ziploc® bag with filter paper

Third lab:  
3 ml sterile water  
buffer solution with diced tumor

**EX. 9: Koch’s Postulates – Methods**

**SPECIAL PRECAUTIONS:** *Agrobacterium tumefaciens* is a plant pathogen which should be handled carefully following laboratory safety rules.

**First lab**
1. Observe diseased plant set up as demo. Record observations in RESULTS section.
2. You will be given a tube containing a diced piece of gall tissue from a diseased carrot
3. Make a smear on a clean slide, using a loopful of liquid from the minced gall and Gram stain. Record observations in RESULTS section.
4. Put another loopful of liquid from the minced gall onto a properly labeled MGY plate and streak for isolation. The plate will be incubated at 25°C for 2-3 days.

**Second lab**
1. Examine the MGY plate for typical *Agrobacterium* colonies: grayish-white and mucoid.
2. Choose one likely colony. Make a smear by mixing part of the colony with a small drop of water and then Gram stain. Record your observations in the RESULTS section. Proceed only if the Gram stain morphology matches results obtained last week.
3. Label the Ziploc® bag with initials & seat #s. Pour 3 ml of sterile water into the bag containing a piece of filter paper to wet the filter paper under the carrot slices.
4. Using a sterile inoculation loop, carefully spread the remainder of the colony on the surface of both of the carrot slices. Carrot slices will be examined after four weeks.

**Third lab**
1. Observe infected carrot slice. Record observations in RESULTS section.
2. Make a smear of the gall material and Gram stain. Record observations in RESULTS section.
3. Discard the carrot and filter paper in the autoclave bag. Stack Petri dishes in the metal coffins on the discard table. Place the razor blade in the metal sharps can.
Exercise 10: Relation of Oxygen to Microbial Growth

Objectives
- Recognize what the following terms mean: aerobe, anaerobe, and facultative anaerobe.
- Employ the use of sodium thioglycollate and resazurin to show the oxygen requirements of bacteria.
- Classify bacteria according to their oxygen requirements.

Reading
Bacteria show considerable variation in their requirement for oxygen. An organism that requires oxygen is called an aerobic; one whose growth is inhibited by oxygen is called an anaerobic; one that can grow under either aerobic or anaerobic conditions is a facultative anaerobe.

In our laboratory exercise we will use a reducing medium called sodium thioglycollate broth. Sodium thioglycollate removes oxygen from the broth media by combining with it chemically. As a result, oxygen is tied-up and is unavailable to the bacteria. However, as might be anticipated in a tube of capped liquid broth, atmospheric oxygen is constantly circulating and dissolving into the broth surface. As the depth in the broth increases, the amount of dissolved atmospheric oxygen diminishes to a point where it can be completely bound by the reducing agent, sodium thioglycollate. Below this point, true anaerobic conditions exist. To show where this occurs, an oxygen sensitive dye, resazurin, is also added to the broth. This dye is colorless when oxygen is not present and pink when oxygen is present.

You should note a pinkish tinge in the upper level of your thioglycollate broth tubes. This shows you how far oxygen has diffused into the medium without being totally bound by the sodium thioglycollate. When performing the following inoculations, be sure the organism is placed well below the bottom of the pink zone.
EX. 10: Oxygen Usage – Materials per Pair

3 sodium thioglycollate broth tubes with resazurin dye
3 PCA soft agar tubes with resazurin
dye broth cultures: *Bacillus subtilis*
   *Proteus vulgaris*
   *Clostridium sp.*

EX. 10: Oxygen Usage - Methods

1. Label the glass portion (NOT the cap!) of the thioglycollate tubes with one name of each of the three bacterial cultures and your seat number.

2. Carefully inoculate each of the tubes with an inoculation needle (not a syringe), going down into the medium about ¾ of the way. Remember to use aseptic technique. One bacterium per tube!

3. **One at a time and working quickly,** carefully inoculate one liquefied PCA+resazurin soft agar tube with a loopful of the appropriate bacterium, then dump the liquefied, inoculated soft agar into the appropriate thioglycollate tube.

4. Place the tubes in the racks at the front table. They will be incubated for 48 hours at 37°C.

5. Next lab, observe the location and appearance of growth in each tube following incubation.

6. Record observations in RESULTS section. First sketch the region of growth for each bacterial type in the diagrams provided; then identify what type of oxygen requirement is shown by such growth.
**Exercise 11: Microbes of the Mouth – Their Relationship to Cavities**

**Objectives**
- Recognize the high numbers and diversity of normal mouth flora.
- Describe the association between *Streptococcus mutans*, *S. salivarius* and *S. sanguis* and cavity production.

**Reading**

The mouth is an exceptionally hospitable environment for microbial growth. Not only is the mouth warm and moist, but a consistent supply of nutrients (food we eat) is present. The mouth also has many different micro-niches where a diverse variety of microbes can flourish. These micro-niches include biofilms made of bacterial cells and extracellular slime which develop on the tongue, teeth, and gums. Some of these bacteria are difficult to grow as they have strict nutritional or atmospheric requirements.

An important member of normal mouth flora is the Streptococcus group of bacteria. Specifically, on the tooth surface, *Streptococcus mutans* is the predominant species, with *S. sanguis* being much less prevalent. On the tongue, *S. salivarius* is a dominant species. All three of these Streptococcus species are capable of metabolizing sucrose (table sugar). Sucrose, a disaccharide, is first broken down by these bacteria into its two monosaccharides, glucose and fructose. The glucose is then combined into a long chain of glucoses, producing a sticky compound called glucan. Glucan coats teeth and is eventually converted into dental plaque as bacteria and other salivary debris combine with the glucan. This dental plaque cannot be washed away by saliva. The fructose and other carbohydrates are fermented to a variety of acids by *S. mutans* and other bacteria in the dental plaque. These acids are capable of eroding tooth enamel, thus leading to cavities. Sucrose is actually the only carbohydrate which can be utilized by bacteria to make glucan. However, all other carbohydrates can be metabolized to produce acids, thus promoting cavity formation. Interestingly, even sugarless candies, those which contain mannitol or sorbitol, can be metabolized to make acids.

We will swab the teeth and culture it on a sucrose agar plate. On sucrose media, *S. mutans*, *S. salivarius* and *S. sanguis* convert the sucrose into glucan, producing colonies which are visibly gummy and sticky. Production of glucan on teeth surfaces is an important initial step in cavity production. To provide a more realistic appreciation for the diversity of this biofilm on the teeth we will place scrapings from the teeth directly on a slide and perform a Gram stain.
**EX. 11: Mouth Microorganisms - Materials per Student**

1 sucrose agar plate
2 sterile cotton swabs

**EX. 11: Mouth Microorganisms - Methods**

1. Take a sterile cotton swab and swab the surface of your teeth for a few minutes. Make a smear without adding water, air dry, and Gram stain. Record observations in the **RESULTS** section. Note: cheek cells will stain as large pink ovals containing darker pink nuclei (fried-egg type appearance). They will be much larger than the bacteria you are looking for, but there might be bacteria clinging to the outside of them.

2. Take a second sterile cotton swab and swab the surface of your teeth again. Swab the first third of a sucrose agar plate using the swab. Streak out the second and third part of the plate with a sterile loop (a loop that has been flamed and cooled), using the streak plate method taught in Exercise four. Label agar side of sucrose plate with your name/seat number and place in appropriate incubation tub, agar-side up. Plates will be incubated at 37°C for 48 h.

3. Next lab, examine your sucrose agar plate. *S. mutans* will form large gummy looking colonies that stick up above the surface of the agar (like tiny gumdrops). Gram stain one colony from your sucrose plate (remember to use a small drop of water when making the smear). Record observations in the **RESULTS** section.
**Exercise 12: Normal Throat Flora**

**Objectives**
- To observe our own normal throat flora.
- To observe the most common type of microbe and its associated hemolysis.
- To realize the diversity of normal throat flora.

**Reading**
In this exercise, we will study the normal bacterial flora of the throat. Normal flora are the organisms found in the throats of healthy people. The throat is a moist, warm environment, allowing many bacteria to flourish. Many different types of organisms are found there. Infection by pathogenic bacteria is actually minimized by the normal flora. That is, bacteria of the normal throat flora suppress the growth of pathogenic bacteria through competition for nutrients and production of inhibitory substances.

*Streptococcus* bacteria are the predominant bacteria found in normal throat cultures. One streptococcal species, *Streptococcus pyogenes*, is a pathogen. It causes strep throat and skin infections as well as several serious diseases including: rheumatic fever, scarlet fever, acute glomerulonephritis, and necrotizing fasciitis.

Streptococci are classified according to the hemolytic reactions they cause while growing on blood-enriched agar. **Hemolysis** is the lysis of red blood cells. If hemolysis occurs, the agar behind the colony will change color or become clear.

Three patterns of hemolysis can occur on blood agar:

1. **Alpha-hemolysis**: Incomplete hemolysis; a green, cloudy zone around the colony.
2. **Beta-hemolysis**: Complete hemolysis; a clear zone around the colony.
3. **Gamma-hemolysis**: No hemolysis; no change in the blood agar around the colony.

Alpha- and gamma-hemolytic streptococci are usually normal flora, whereas beta-hemolytic streptococci are often pathogens. *Streptococcus pyogenes* produces beta hemolysis.
EX. 12: Throat Microorganisms – Materials per Student

1 blood agar plate
1 sterile cotton swab
1 sterile tongue depressor

EX. 12: Throat Microorganisms - Methods

1. Swab the back of your throat with a sterile cotton swab. The area to be swabbed is between the golden arches (glossopalatine arches). Do not hit the tongue, which should be held down with a sterile tongue depressor.

2. Use the swab to inoculate one-third of a blood agar plate. Streak out the second and third part of the plate with a sterile loop, using the streak plate method taught in Exercise 4. Label agar side of blood agar plate with your name/seat number and place in the appropriate incubation tub. Plates will be incubated at 37°C for 48 hours.

3. Next lab, observe colony morphologies and type(s) of hemolysis on your blood agar plate. Observe hemolysis by holding the plate up to the light, lid-side up, and observing the area immediately surrounding colonies.

4. Select a colony from your plate and perform a Gram stain.

5. It is not possible to identify microorganisms reliably without exact biochemical tests. You may, however, make a presumptive identification of one of your bacteria based on your Gram stain results and colony morphology. Use Table 13.1 as a reference.

6. Record observations in the RESULTS section.

TABLE 13.1: COMMON NORMAL FLORA OF THE THROAT

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gram reaction/morphology</th>
<th>Colony description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraxella catarrhalis</td>
<td>Gram- diplococci/diplobacilli</td>
<td>Large, smooth, gray</td>
</tr>
<tr>
<td>Candida (yeast)</td>
<td>Gram+ spheres (large)</td>
<td>Large, smooth, pasty, cream/off-white</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>Gram+ bacilli/coccobacilli</td>
<td>Large, rough, white</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>Gram+ bacilli (slender)</td>
<td>Medium, smooth, cream</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>Gram+ streptococci</td>
<td>Small, smooth, translucent white/grey</td>
</tr>
<tr>
<td>Haemophilus sp.</td>
<td>Gram- coccobacilli (tiny)</td>
<td>Small, smooth, transparent</td>
</tr>
<tr>
<td>Neisseria sp.</td>
<td>Gram- diplococci</td>
<td>Small, smooth, grey or yellowish</td>
</tr>
</tbody>
</table>
Exercise 13: Testing Water for Pathogenic Microorganisms

Objectives

- Practice the steps involved in testing water for purity.
- Identify coliform bacteria.

Reading

Water can be a serious carrier of disease. Municipal systems in this country treat their water with chlorine or ozone to kill microorganisms; however, water must still be routinely checked for the presence of potential pathogens. Pathogens can be difficult to grow and for this reason an indicator organism is used instead. The indicator organism must grow rapidly, be present if pathogens are present and die off at the same rate as pathogens. In this country, the indicator organisms used are coliform bacteria. Coliforms are Gram negative rods which grow facultatively and ferment lactose with the production of gas at 35°C in 48 hours.

LT Broth with Durham Tube: The preliminary test for coliforms uses lactose tryptone (LT) broth and a Durham tube to trap any gas produced. The coliform group contains several types of bacteria. One important member is Escherichia coli, a bacterium found in the large intestine of most warm blooded animals. If E. coli is present, there is a high chance that fecal contamination of the water has occurred and it is unfit to drink. E. coli and organisms which test as very similar are called fecal coliforms.

EMB Plates: We will test for the presence of fecal coliforms using EMB (eosin methylene blue) agar plates. This media inhibits Gram positive organisms because it contains toxic dyes which can be absorbed through their porous cell wall. It also contains lactose. Organisms which ferment lactose, producing acid, will cause the dye in the surrounding media to crystallize, forming readily visible green metallic-looking colonies.

An alternative test involves growth of organisms in EC + MUG broth. EC is an abbreviation for E. coli. The original EC broth was developed to improve the detection of the coliform group and E. coli in water and other sources such as waste water, shellfish and food. Further research found that adding 4-methylumbelliferyl-β-D-glucuronide or MUG to the EC broth can be used to distinguish E. coli from other bacteria in the coliform group. E. coli produces an enzyme called glucuronidase that converts MUG into a fluorescent product that glows in the dark when a long-wave (366nm) UV light is used. All coliforms grown in the broth will have turbidity and produce gas but only E. coli will glow under the UV light.
EX. 13: Water and Coliforms – Materials per Pair

Lab 1
100 ml of 2X lactose tryptone broth with Durham tube
100 ml of water sample

Lab 2
1 EMB agar plate
1 short sterile transfer pipette

EX. 13: Water and Coliforms - Methods

Lab 1
1. Add 100 ml of water sample to 100 ml of 2X (i.e., double strength) lactose tryptone broth containing a Durham tube. Do not shake the bottle – you don’t want to introduce a bubble into the Durham tube.

2. Label the bottle with your seat numbers and water sample number. Place in the appropriate incubation tub. Bottles will be incubated at 37°C for 48 hours.

Lab 2
1. Examine broth for turbidity or sediment at the bottom, which indicates microbial growth. Examine Durham tube for presence of gas, indicated by a bubble trapped in the tube. Only coliforms will produce gas from lactose. Record observations in RESULTS section.

2. Gently swirl the bottle to redistribute the bacteria that may have settled to the bottom of the bottle. Using a sterile inoculation loop, Collect a loopful of water sample and perform a T-streak on EMB plate.

3. Label the plate with name/seat number and water sample number. Tubes will be incubated at 37°C for 48 hours.

Lab 3
1. Examine the EMB plates for growth. Non-fecal-coliforms will produce dark pink-purple colonies, while fecal coliforms produce colonies with a green metallic sheen.

2. Record observations in RESULTS section.
Exercise 14: Conjugation and the Transfer of Antibiotic Resistance

Objectives
• Identify the following terms: conjugation, plasmid, pili/pilus, F⁺ (donor), F⁻ (recipient).
• Observe how genes carried on either a chromosome or plasmid can affect the antibiotic resistance of a bacterium.

Reading
Bacteria have several natural mechanisms for exchanging genetic material. The natural mechanism we will study is called conjugation. In conjugation, a self-replicating extrachromosomal, circular piece of DNA called a plasmid is passed from one strain of bacteria, the donor (F⁺), to another strain of bacteria, the recipient, (F⁻), through a hollow tube called the sex pilus. The plasmid DNA contains genes that can then be expressed in the recipient bacterial cell. In our exercise, the donor strain of Escherichia coli carries genes for resistance to one antibiotic on its plasmid. The recipient strain of E. coli naturally has a gene for resistance to a second antibiotic on its DNA chromosome. After conjugation between the recipient and donor strains of E. coli, a new strain of E. coli is produced, which should have resistance to both antibiotics. Note that by gaining the plasmid, this new E. coli has been converted into an F⁺ type of bacterium.

The diagram on the next page shows the process of conjugation involving plasmid transfer.
Transfer of Antibiotic Resistance by Conjugation

*E. Coli* strain with plasmid (F\(^+\))

![Diagram showing the transfer of plasmid from F\(^+\) strain to F strain.]

*E. Coli* strain without plasmid (F)

![Diagram showing the transfer of plasmid from F\(^+\) strain to F strain.]

Copy of plasmid transferred.

![Diagram showing the transfer of plasmid from F\(^+\) strain to F strain.]

Chromosome (blue) plasmid

Chromosome (red)
EX. 14: Conjugation – Materials per Pair

1 ml of *Escherichia coli* HT 99 (Donor)
1 ml of *Escherichia coli* J-53R (Recipient)

1 chloramphenicol (C) plate
1 rifampicin (R) plate
2 chloramphenicol + rifampicin (C+R) plates

1 sterile long transfer pipette
1 sterile short transfer pipette
1 hockey stick and ethanol (EtOH)

EX. 14: Conjugation - Methods

1. Divide one chloramphenicol (C) plate, one rifampicin plate (R), and one chloramphenicol + rifampicin (C+R) in half by drawing a line with your pen on the bottom surface of the plate. Label one half ‘HT-99’ and one half ‘J-53R’ for each plate. Also label with your names/seat numbers. Set your remaining C+R plate aside until later.

2. Aseptically inoculate one loopful of *E. coli* HT-99 culture on the appropriate half of each plate (C plate, R plate, and C+R plate). Don’t worry about streaking for isolation, just streak a zigzag line of culture down one half of the plate.

3. Aseptically inoculate one loopful of *E. coli* J-53R culture on the other half of each plate, with a streak as before.

4. Pipette the remaining culture from the large tube into the culture contained in the small tube; swirl the small tube and set tube aside for at least 20 minutes without disturbing. At the end of the 20 minutes, swirl gently.

5. Pipette 0.5 ml of the mixed culture (HT-99 plus J-53R mixture) on your remaining chloramphenicol + rifampicin plate. Use a sterile hockey stick to spread the liquid. Do not invert the plate! Place in the appropriate incubation tub, lid-side up. All plates will be incubated at 37°C for 48 hours.

6. Next lab, examine all plates for growth and record observations in the **RESULTS** section.

**Growth on an antibiotic-containing medium means that the bacterial strain is resistant to the antibiotic(s) in that medium. Non-growth on an antibiotic-containing medium means that the bacterial strain is susceptible (non-resistant) to the antibiotic(s) in that medium.**

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Exercise 15: *Staphylococcus aureus* in Potato Salad – One Cause of Food Poisoning

Objectives

- Prepare a food, potato salad, and mishandle it to provide conditions for *Staphylococcal* contamination and growth.
- Analyze the food for the presence of *S. aureus* and determine the level of contamination.

Reading

*Staphylococcus aureus* is one of the most common causes of food-borne poisoning in the United States, producing an illness called Staph food poisoning. Not all strains of *S. aureus* cause food poisoning. Only those strains that can make poisons known as enterotoxins lead to food-borne illness. Such *S. aureus* strains usually get into the foods due to food handlers. Many food handlers have *S. aureus* on their hands and in their mouths or noses as part of their normal flora. Thus, when touching food during preparation, or by coughing and/or sneezing on food, food handlers inoculate *S. aureus* into the food. Food poisoning results if the *S. aureus* in such foods remains at a temperature that allows it to grow and produce enterotoxins. Staph food poisoning results from consumption of the enterotoxin, rather than consumption of the bacteria themselves. Subsequent heating of the food can destroy the bacteria but will not inactivate the toxin. The foods most often involved in Staph food poisoning are those which are handled during their preparation, have rich sources of **protein and fat, and have a high salt content**. Specific foods include creamy salads, such as potato salad, cream-filled pastries, and salty meats, such as ham. Foods that are suspected of causing Staph food poisoning often have large numbers of *S. aureus*. To detect and enumerate these bacteria, one must perform a **dilution** before plating a sample to obtain an accurate count.

In this exercise, students will prepare potato salad in a deliberately unsanitary manner. The potato salad will also be allowed to remain at a warm temperature (37°C) for a period of several hours. Similar poor temperature control often happens with potato salad taken on picnics (where many cases of Staph food poisoning occur). The potato salad will then be diluted and cultured as described in the procedure section. Students will then be able to determine the actual number of *S. aureus* which grew in their potato salad.

An excellent media that selects for staphylococci is **mannitol salt agar (MSA)**. The high (7.5%) salt concentration in this media inhibits all skin bacteria except staphylococci. An additional ingredient of this media, the sugar **mannitol**, helps differentiate between the two main staphylococci species, *S. aureus* and *S. epidermidis*. *S. aureus* ferments mannitol producing **acid**. MSA media also contains **phenol red**, which is pink under neutral or alkaline pH but turns yellow in the presence of acid. If *S. aureus* is present it will grow on the MSA plate and ferment mannitol producing acid that will turn the agar yellow. If mannitol is not fermented the media will remain pink.
EX. 15: Potato Salad – Materials per Group of 4

Lab 1:
- 2 small boiled potatoes
- 1 boiled egg
- 1 food container
- butcher paper
- Salt, mayonnaise

Lab 2:
- 1 x 99 ml diluent bottle
- 3 x 9 ml diluent tubes
- 2 sterile short transfer pipettes
- 4 mannitol salt agar (MSA) plates
- 1 hockey stick, flaming EtOH
- 1 tongue depressor

EX. 15: Potato Salad - Methods

Lab 1 - Preparation of Potato Salad - work in groups of 4
1. DO NOT WASH YOUR HANDS BEFORE BEGINNING.

2. Using bare hands, mash the cooked potatoes into small pieces into the paper tub. Peel the egg and add it to the potatoes in a similar manner.

3. Add approximately 1 teaspoon of salt and 2-3 tablespoons of mayonnaise.

4. Mix the salad with bare hands, with all group members taking a turn at mixing. Try not to be too clean.

5. Label the food container with a group name, the lab day and time (e.g M 10:30) and with the word TOXIC. Containers will be incubated at 37°C for 4 hours. The product will be refrigerated after that point, mimicking a potato salad at a park that is subsequently brought home and refrigerated.

Lab 2 – Serial Dilution of Potato Salad Bacteria – DO NOT TASTE PRODUCT
1. Prepare serial dilutions of the potato salad by removing approximately 1 g (¼ tsp.) from the center of the container and adding it to a 99 ml bottle of diluent. Avoid chunks of potato and try to get some of the softer or more liquid parts of the potato salad.

2. Tightly cap the bottle and shake vigorously for 30 sec. This represents a 1:100 or $10^2$ dilution of the potato salad. Label this bottle $10^2$. Using a sterile pipette, transfer 1 ml of the $10^2$ dilution to a 9 ml diluent tube and mix well. This new dilution represents a 1:10 dilution of the 1:100 dilution, for a final dilution of $10^3$. Label this tube $10^3$. 
3. **Using the same pipette** from step 2, transfer 1 ml of the $10^3$ dilution to a new 9 ml diluent tube and mix well. This new dilution represents a 1:10 dilution of the $10^3$ dilution for a final dilution of $10^4$. Label this tube $10^4$.

4. **Using the same pipette** from step 3, transfer 1 ml of the $10^4$ dilution to a new 9 ml diluent tube and mix well. This new dilution represents a 1:10 dilution of the $10^4$ dilution for a final dilution of $10^5$. Label this tube $10^5$.

5. Label the MSA plates with seat #s and **final dilutions**. Use a **NEW sterile pipette** to plate the following dilutions of potato salad on mannitol salt agar (MSA) plates in the order listed below:

- 0.5 ml of the $10^5$ dilution on the 1st plate (1:100,000 dilution of potato salad bacteria)
- 0.5 ml of the $10^4$ dilution on the 2nd plate (1:10,000 dilution of potato salad bacteria)
- 0.5 ml of the $10^3$ dilution on the 3rd plate (1:1000 dilution of potato salad bacteria)
- 0.5 ml of the $10^2$ dilution on the 4th plate (1:100 dilution of potato salad bacteria)

6. Spread the diluted potato salad over the plates with a flame-sterilized hockey stick. Do not invert MSA plates. Incubate the plates at $37^\circ$C for 48 hours.

**Lab 3 - Microbiological Examination**

1. Count the colonies on the plates. Optimum plates for further calculations will have a total of 25-250 colonies. Colonies that form on MSA plates typically represent a species from the *Staphylococcus* genus. *Staphylococcus aureus* colonies will form a yellow halo around them. If enough colonies of *S. aureus* are on a single plate they can change the entire plate yellow.

2. Using the colony counts, dilution values and volume plated calculate the number of *Staphylococcus* (not just *S. aureus*) per gram (Staph/g) of potato salad (1 ml ≈ 1 g).

   Calculation: (number of colonies counted on plate multiplied by the final dilution factor of the plate) divided by volume plated equals the number of *Staphylococcus* colony-forming units (CFU) per gram of potato salad.

   For example, if the colony counts of Staph are 78 CFU on the $10^3$ dilution plate then:
   
   \[
   \frac{(78 \text{ CFU} \times 10^3)}{0.5 \text{ ml}} = 78000 \text{ CFU}/0.5 \text{ ml} = 156000 \text{ CFU/ml}
   \]
   
   or $1.56 \times 10^5$ Staph CFU/gram of potato salad

3. Select one colony on an MSA plate to Gram stain, to verify the presence of *Staphylococcus*. Record your observations in the **RESULTS** section.
Exercise 16: Microbial Metabolism – Fermentation of Carbohydrates

Objectives

• Describe the breakdown products of glucose, lactose, and sucrose carbohydrates.
• Interpret carbohydrate tubes and the fermentation products produced.

Reading

Metabolism can be defined as the sum total of all chemical reactions which occur inside the cell. The metabolism of carbohydrates entails the catabolism (or breakdown) of certain large molecules, such as disaccharides (double sugars), into smaller ones called monosaccharides (single sugars). For such breakdown to occur, enzymes are required. In this exercise the 2 disaccharides, sucrose and lactose, are broken down by the enzymes sucrase and lactase respectively. These reactions proceed as follows:

\[
\text{Lactose (disaccharide)} \rightarrow \text{Glucose + Galactose (two monosaccharides)} \\
\text{Sucrose (disaccharide)} \rightarrow \text{Glucose + Fructose (two monosaccharides)}
\]

The monosaccharides are then further degraded by a combination of glycolysis and one of two processes, fermentation or respiration. They are fermented by some bacteria to yield organic acids, alcohols and/or gases. Fermentation reactions also release a small number of energy molecules (such as ATP) which are needed to "fuel" other bacterial chemical reactions. Other organisms process monosaccharides more completely. In a process known as aerobic respiration, they produce abundant ATP, water, and CO₂ gas. Aerobic respiration takes place only when O₂ is available.

This exercise illustrates a simple method to detect acid and gas formation from carbohydrate breakdown. Formation of acids is detected by including a pH indicator in microbial growth media. In this experiment, the sugar broths contain the pH indicator bromcresol purple (BCP). This pH indicator is purple at pH 6.8 (near neutral pH) and yellow at pH 5.2 (acid pH). In these broths, gas formation is detected by the use of a small inverted tube referred to as a Durham tube.
EX. 16: Metabolism and Fermentation – Materials per Pair

3 tubes of glucose-BCP broth with Durham tubes (red caps)
3 tubes of sucrose-BCP broth with Durham tubes (blue caps)
3 tubes of lactose-BCP broth with Durham tubes (green caps)

broth cultures:
  *Bacillus subtilis*
  *Micrococcus luteus*
  *Klebsiella pneumoniae*

EX. 16: Metabolism and Fermentation - Methods

1. Inoculate a different tube of each medium with a loopful of *Bacillus subtilis*, *Micrococcus luteus*, and *Klebsiella pneumoniae*. Use aseptic technique throughout! If even a small amount of bacteria or sugar gets transferred to the next tube, it will screw up the results!

   *Bacillus subtilis*: inoculate one tube each of glucose, sucrose and lactose.
   *Micrococcus luteus*: inoculate one tube each of glucose, sucrose and lactose.
   *Klebsiella pneumoniae*: inoculate one tube each of glucose, sucrose and lactose.

   Note: Be sure to label each tube on the glass with name of bacterium, your names/seat numbers, and make certain you replace the cap on the same tube. Place tubes into rack in appropriate incubation tube. All tubes will be incubated at 37°C for 48 h.

2. Next lab period, observe the cultures for both acid and gas production.

3. Record observations in the RESULTS section.
Exercise 17: Cultured Dairy Products

Objectives
• Observe microorganisms which are used to produce cultured dairy products.
• Compare the properties of different ripened cheeses.
• Acquire knowledge about the types of changes produced by the microbial metabolism of milk which result in traditional cultured dairy products.

Reading
Production of cultured dairy products relies on a variety of microorganisms depending on the product being made. Lactic acid bacteria are the most frequently utilized. They ferment carbohydrates to produce lactic acid.

Milk/Yogurt - This traditional European dairy product is made of pasteurized or boiled milk. Two lactic acid bacteria, Streptococcus thermophilus and Lactobacillus bulgaricus are used together during incubation at high temperature (45°C). Boiling and the temperature of incubation help prevent the growth of unwanted contaminants. S. thermophilus produces lactic acid from the milk sugar lactose. This encourages the growth of L. bulgaricus which produces additional acid and proteolytic enzymes. Milk proteins denature causing the milk to solidify. The milk develops a distinctive tangy flavor due to the fermentation products like lactic acid. The pH of the milk drops significantly because of the production of lactic acid.

Cheese - Many types and sources of milk are used all over the world to produce cheese. Additional variety is provided by the use of different culturing agents. Lactococcus lactis and species from the genus Leuconostoc are often used for the initial culture. Lactic acid produced by the bacteria, along with the enzyme rennin which is added separately, curdles the milk separating the curds (milk solids) from the whey (liquid). Additional microorganisms called ripening agents can be added to the curd. The final taste and texture of the cheese can be modified by the length and conditions of the ripening period.

<table>
<thead>
<tr>
<th>Type</th>
<th>Cheese</th>
<th>Example Ripening agent(s)*</th>
<th>Ripening Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft:</td>
<td>Brie</td>
<td><em>Penicillium camemberti</em></td>
<td>4-6 months</td>
</tr>
<tr>
<td></td>
<td>Camembert</td>
<td><em>Brevibacterium linens</em></td>
<td>4-6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Penicillium camemberti</em></td>
<td></td>
</tr>
<tr>
<td>Semi-soft:</td>
<td>Blue</td>
<td><em>Penicillium roqueforti</em></td>
<td>3-6 months</td>
</tr>
<tr>
<td></td>
<td>Limburger</td>
<td><em>Brevibacterium linens</em></td>
<td>1-2 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Micrococcus sp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Mixed yeast</em></td>
<td></td>
</tr>
<tr>
<td>Hard:</td>
<td>Cheddar</td>
<td><em>Lactococcus lactis</em></td>
<td>4-12 months</td>
</tr>
<tr>
<td></td>
<td>Swiss</td>
<td><em>Leuconostoc sp.</em></td>
<td>3-12 months</td>
</tr>
<tr>
<td></td>
<td>Parmesan</td>
<td><em>Propionibacterium sp.</em></td>
<td>24-48 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Streptococcus thermophilus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lactobacillus bulgaricus</em></td>
<td></td>
</tr>
</tbody>
</table>
EX. 17: Dairy Products – Materials per Pair

- traditional Blue, Cheddar, and Limburger cheeses
- boiled milk fortified with 3-5% powdered skim milk, held at 45°C in waterbath
- commercial live culture yogurt
- pH paper

EX. 17: Dairy Products - Methods

Lab 1
Yogurt:
In pairs, test the initial pH of a tube of fortified boiled milk. Use pH paper and the color guide to determine the pH. Using a sterile loop inoculate the milk with starter culture (live culture commercial yogurt). Use several loopfuls. Roll the inoculated tube back and forth in your hand to mix and label it on the glass with your names/seat #s. Return the tube to the 45°C water bath. The yogurt will be incubated for about 4 hours and then refrigerated.

Cheese:
Examine the Blue cheese. You may note long straight holes drilled into the curd to provide the secondary culture agent Penicillium roqueforti with the oxygen it requires. The blue veins of the cheese are made of spores which develop from the mold’s hyphal filaments. A wet mount demo has been prepared by scraping some blue material from the cheese block and adding a drop of distilled water. Examine this wet mount, which has been placed under the 40/45X objective. Look for hyphal filaments or spores. Remember, the 100X oil immersion objective is not used for a wet mount!

Examine the Cheddar and Limburger cheeses. Limburger has a particularly characteristic odor. Use a little water to prepare smears of both types of cheese. Try to get some of the surface material (the rind) from the Limburger cheese – most of the microbes are located here. Gram stain your smears and examine them. You may find that de-staining the smears takes longer than usual due to the fat content of the cheese. Record all observations in the RESULTS section.

Lab 2
Yogurt:
Examine the tube of milk for consistency and odor. Test the final pH using pH paper. Prepare a smear of the yogurt and Gram stain. (Lactic Acid Bacteria responsible for making yogurt are typically Gram positive). Record observations in the RESULTS section.
Exercise 18: The Fungi – Molds and Yeast

Objectives
• Recognize the difference between filamentous fungi and non-filamentous fungi.
• Identify the macroscopic and microscopic features of common molds and yeast.

Reading
The study of fungi is referred to as mycology (Gr. "Mykes" = fungus). **Fungi** are a group of eukaryotic microorganisms which live by digesting organic material. Fungi can be distinguished from algae because the fungi do not have chlorophyll and thus cannot carry out photosynthesis, even if some appear green in color. Fungi can be differentiated from protozoa in that most are non-motile and have a distinct cell wall. Fungi can be differentiated from bacteria, which are prokaryotes, by the fact that fungal cells are much larger and have nuclei and other organelles typical of eukaryotic cells. Although the fungi are a large and rather diverse group of eukaryotic organisms, two examples of fungi are examined in this exercise: molds and yeast.

**Molds** are filamentous fungi which everyone has observed growing on stale bread, cheese or fruit. The filaments, called vegetative **hyphae** (Gr. "Hyphe" = web) usually grow together across a surface to form a compact mat, collectively called a **mycelium**. The continual branching and intertwining of fungal filaments results in the formation of a visible structure on the surface of the substrate. A portion of the mycelium becomes aerial (**aerial hyphae**) and gives rise to specialized cells at its tips. This ‘fuzzy’ growth is what we see and call mold. These specialized cells are referred to as **spores**. They allow the mold colony to reproduce asexually, searching out new locations and nutrients. Spores are extremely lightweight and very easily dispersed by even the slightest air current (such as opening the lid quickly from a Petri dish). This motion scatters them about, disseminating the mold throughout the area. The color of a particular mold is due largely to the spores, which may be pigmented. Depending on the division to which a mold belongs the asexual spore carrying structure may be called a conidiophore or a sporangiophore. A conidiophore gives rise to **conidia** on the outside of the aerial hyphae, while a sporangiophore gives rise to **sporangia** contained within the aerial hyphae. These are used as characteristics for identification of individual mold species. Molds are additionally identified by whether their hyphae may be described as **septate** or **aseptate** (or coenocytic), based on whether or not the hyphae contain cross walls or partitions between cells. Molds are used for many industrial purposes as well as causing some human diseases.

**Yeast** are **unicellular** (single cell) fungi generally distinguished from molds by their lack of true filaments. The yeast have a unique type of asexual reproduction called **budding**. In this process a small, new cell (called the bud or daughter cell) is pinched off from the mother cell. Yeast are extremely important to us. An example of a useful yeast which is responsible for production of bread, beer and wine is **Saccharomyces cerevisiae**. Other yeast can be pathogenic causing such diseases as vaginal infections or thrush, a fungal infection of the mucous membranes of the mouth.
EX. 18: Fungi - Materials

potato dextrose agar (PDA) plates of:  
\( Aspergillus \) sp.  
\( Penicillium \) sp.  
\( Rhizopus \) sp.

stained slides showing microscopic structure of above molds  
suspension culture of the yeast: \( Saccharomyces cerevisiae \)

EX. 18: Fungi - Methods

Note: the materials needed for this lab are set up as demonstrations around the room.

1. Observe the molds on the plates as they appear under the dissecting microscopes. Note the color (pigment) and general appearance of the spores.

2. Examine the prepared slides of the molds under the light microscopes. Look closely at individual hyphal filaments to determine whether each mold is septate or non-septate. Look closely at aerial hyphae to determine whether each mold forms conidia (outside aerial hyphae) or sporangia (inside aerial hyphae).

3. Observe the wet mount of the \( Saccharomyces cerevisiae \) suspension and note the morphology of the yeast cells. Look for individual cells that are budding.

4. Draw your observations in the RESULTS section.
MB230 Introduction to Microbiology

Results

Name: ________________________________

Section: ______________________________

Room Number: _________________________

Seat Number: __________________________
EX. 1: Use of the Microscope – Results

Part A. Unstained wet mounts. Draw what you see using the **10X or 40/45X objective**.

Hay Infusion Broth (protozoa and bacteria)

Pond Water (algae and bacteria)

Part B. Stained permanent slides. Draw what you see using the **100X objective**.

*Staphylococcus aureus* (bacterium)

*Candida albicans* (yeast)

*Bacillus subtilis* (bacterium)

TA Initials:
EX. 2: Microorganisms in the Air – Results

Information from PCA plate exposed to air:

Number of different bacteria and yeast colonies ________________
(identified by varying color, shape, texture, size, etc)

Number of different mold colonies ________________
(identified by varying color, shape, texture, size, etc)

Choose one colony on your agar plate and describe it to the best of your ability using table 2.1. Do not remove the lid of the Petri dish, except for brief periods of time.

<table>
<thead>
<tr>
<th>Size</th>
<th>Pinpoint</th>
<th>Small</th>
<th>Moderate</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigmentation</td>
<td>Color of colony</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Form (shape of the colony)</td>
<td>Circular: round, even colonies</td>
<td>Irregular: irregularly shaped colonies</td>
<td>Rhizoid: rootlike, spreading growth</td>
<td></td>
</tr>
<tr>
<td>Margin (appearance of colony outer edge)</td>
<td>Entire: sharply defined, even</td>
<td>Lobate: marked indentations</td>
<td>Undulate: wavy indentations</td>
<td>Serrate: toothlike appearance</td>
</tr>
<tr>
<td>Elevation (degree to which colony growth is raised on agar surface)</td>
<td>Flat: elevation not discernible</td>
<td>Raised: slightly elevated</td>
<td>Convex: dome shaped elevation</td>
<td>Umbonate: raised, with center of colony markedly elevated</td>
</tr>
</tbody>
</table>

TA Initials:
EX. 3: Morphology – Results

EXAMINATION OF STAINED CELLS – Draw a few representative cells that you observe. Make your drawings sufficiently large.

\[ \text{TA Initials:} \]

\[ \text{Staphylococcus epidermidis} \quad \text{Escherichia coli} \]

\[ \text{colony from PCA plate (Ex.2)} \quad \text{Spirillum (permanent slide)} \]
EX. 4: Streak Plating – Results

Draw the bacterial growth that you observe on your streak plate:

How many different COLONY types do you observe?

How many isolated colonies do you observe?
EX. 5: Environmental Sampling – Results

RODAC Plate:

<table>
<thead>
<tr>
<th>Surface Sampled</th>
<th>Number of Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Colony description of the most common colony type on RODAC plate:

Do you have acceptable sanitation? (Plates with less than 100 colonies.)

Gram reaction: ______________________

Cell morphology: _____________________

TA Initials:

Swab Technique (PCA plate):

<table>
<thead>
<tr>
<th>Surface Sampled</th>
<th>Number of Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Colony description of the most common colony on PCA plate:

Do you have acceptable sanitation? (Plates with less than 100 colonies.)

TA Initials:
EX. 6: Gram Stain - Results

**Micrococcus luteus**
- TA Initials: __________________
  - Morphology: _____________
  - Gram reaction: ___________

**Escherichia coli**
- TA Initials: __________________
  - Morphology: _____________
  - Gram reaction: ___________

**TSA plate colony**
- □ yellow colony or
  - □ white colony
- TA Initials: __________________
  - Morphology: _____________
  - Gram reaction: ___________

**TSA plate colony bacterial species:** ________________________________
EX. 7: Nitrogen Usage – Results

Observations of plants:

<table>
<thead>
<tr>
<th>Group</th>
<th>Color</th>
<th>Vigor/Health</th>
<th>Height (cm)</th>
<th>Nodules (y/n?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gram reaction: ______________________

Cell morphology: ____________________
EX. 8: Bacteriophage – Results

Determine how many bacteriophage were present per ml of the original culture by multiplying the number of plaques on the plate by the dilution factor.

\[
\frac{\text{plaque count} \times \text{dilution factor}}{\text{volume of virus plated}} = \text{PFU (plaque-forming unit)/ml}
\]

Work with the rest of your table to fill in the chart below and estimate the original phage concentration. Plates with between 25 and 250 plaques give the best statistical estimate of the original population. Record completely cloudy plates as zero or no plaques.

1. How many plaques are on the plate made by you and your partner?

2. What is your dilution factor?

3. Fill out the following chart, using plate counts from the entire dilution series:

<table>
<thead>
<tr>
<th>Plaque Count</th>
<th>Dilution Factor</th>
<th>Volume of Virus Plated</th>
<th>PFU/mL Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 on tube = $10^4$</td>
<td></td>
<td>0.1 mL</td>
<td></td>
</tr>
<tr>
<td>5 on tube = $10^5$</td>
<td></td>
<td>0.1 mL</td>
<td></td>
</tr>
<tr>
<td>6 on tube = $10^6$</td>
<td></td>
<td>0.1 mL</td>
<td></td>
</tr>
<tr>
<td>7 on tube = $10^7$</td>
<td></td>
<td>0.1 mL</td>
<td></td>
</tr>
<tr>
<td>8 on tube = $10^8$</td>
<td></td>
<td>0.1 mL</td>
<td></td>
</tr>
<tr>
<td>9 on tube = $10^9$</td>
<td></td>
<td>0.1 mL</td>
<td></td>
</tr>
</tbody>
</table>

4. Estimate of original phage concentration (PFU/ml), using most accurate plaque count(s):

TA Initials: 57
EX. 9: Koch’s Postulates - Results

Lab 1

1. Describe appearance of control carrot:

2. Describe appearance of diseased carrot:

3. Gram stain of *A. tumefaciens* from minced plant tissue (gall):
   
   Gram reaction: ____________
   
   Cell morphology: ____________

Lab 2

1. Describe colony morphology on MGY agar plate:

2. Gram stain of *A. tumefaciens* colony from MGY agar plate:
   
   Gram reaction: ____________
   
   Cell morphology: ____________

Lab 3

1. Describe appearance of control carrot:

2. Describe appearance of inoculated carrot:

3. Gram stain of *A. tumefaciens* from minced plant tissue (gall):
   
   Gram reaction: ____________
   
   Cell morphology: ____________
EX. 10: Oxygen Usage – Results

1. Draw where each bacterium grew in the thioglycollate tubes:

   - Bacillus subtilis
   - Proteus vulgaris
   - Clostridium sp.

   TA Initials:
EX. 11: Mouth Microorganisms - Results

Lab 1:
Gram stain of direct smear of teeth:

<table>
<thead>
<tr>
<th>Colony</th>
<th>Colony Description</th>
<th>Approx. % of total colonies on plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lab 2:
1. Describe up to three different colony types found on your sucrose plate (size, color, texture, etc.):

2. Gram stain of one colony from sucrose agar plate:

Gram reaction: _______________  
Cell morphology: _______________
EX. 12: Throat Microorganisms - Results

1. Describe the different colony types on your blood agar plate:

<table>
<thead>
<tr>
<th>Colony #</th>
<th>Description of Colony</th>
<th>Hemolysis type</th>
<th>Approx. % of total colonies on plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. What type of hemolysis is most prevalent on your blood agar plate?

3. Gram stain of one colony from blood agar plate:

   Colony #:________
   Gram reaction: ________________
   Cell morphology:______________

3. Presumptive identification of the organism, using Table 11.1:

**TABLE 13.1: COMMON NORMAL FLORA OF THE THROAT**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gram reaction/morphology</th>
<th>Colony description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraxella catarrhalis</td>
<td>Gram – diplococci/diplobacilli</td>
<td>Large, smooth, gray</td>
</tr>
<tr>
<td>Candida (yeast)</td>
<td>Gram + spheres (large)</td>
<td>Large, smooth, pasty, cream/off-white</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>Gram + bacilli/coccobacilli</td>
<td>Large, rough, white</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>Gram + bacilli (slender)</td>
<td>Medium, smooth, cream</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>Gram + streptococci</td>
<td>Small, smooth, translucent white/grey</td>
</tr>
<tr>
<td>Haemophilus sp.</td>
<td>Gram – coccobacilli (tiny)</td>
<td>Small, smooth, transparent</td>
</tr>
<tr>
<td>Neisseria sp.</td>
<td>Gram - diplococci</td>
<td>Small, smooth, grey or yellowish</td>
</tr>
</tbody>
</table>
EX. 13: Water and Coliforms – Results

Lab 2:

1. Is growth present in the lactose tryptone broth?

2. Is gas present in the lactose tryptone broth?

3. Is your organism a coliform or non-coliform?

Lab 3:

4. Is growth present on the EMB plate?

5. Does the growth have a green metallic sheen?

6. Is your organism a fecal coliform and non-fecal coliform or a non-coliform?
EX. 14: Conjugation – Results

Fill in the chart below (growth or non-growth):

<table>
<thead>
<tr>
<th>Plate with:</th>
<th>chloramphenicol</th>
<th>rifampicin</th>
<th>chloram. + rifamp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“New”:</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

TA Initials: 63
EX. 15: Potato Salad – Results

Colony Count of MSA plates

<table>
<thead>
<tr>
<th>Colony Count</th>
<th>Dilution</th>
<th>Calculation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^2$</td>
<td>(___ x $10^2$)/0.5 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^3$</td>
<td>(___ x $10^3$)/0.5 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^4$</td>
<td>(___ x $10^4$)/0.5 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^5$</td>
<td>(___ x $10^5$)/0.5 ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. What is your final estimate of *Staphylococcus* per gram (*Staph/g*) of your potato salad? Show your work.

2. Based on the MSA colony growth, approximately what percentage of this is *Staphylococcus aureus*?

3. Gram stain of one colony from an MSA plate:

   Gram reaction: ______________

   Cell morphology: ______________

   TA Initials:
**EX. 16: Fermentation – Results**

Record the results of the carbohydrate sugar fermentation as negative (-) fermentation; **A** for acid fermentation (broth changes to yellow); or **AG** for acid and gas fermentation (broth changes to yellow and bubble present in Durham tube).

<table>
<thead>
<tr>
<th>BACTERIUM</th>
<th>GLUCOSE (red)</th>
<th>SUCROSE (blue)</th>
<th>LACTOSE (green)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TA Initials:
### EX. 17: Dairy Products – Results

<table>
<thead>
<tr>
<th>Cheese Type</th>
<th>Odor</th>
<th>Consistency</th>
<th>Microscopic Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Cheese</td>
<td></td>
<td></td>
<td>Total mag. (wet mount) =</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Description:</td>
</tr>
<tr>
<td>Cheddar</td>
<td></td>
<td></td>
<td>Total mag. (Gram stain) =</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Description:</td>
</tr>
<tr>
<td>Limburger</td>
<td></td>
<td></td>
<td>Total mag. (Gram stain) =</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Description:</td>
</tr>
</tbody>
</table>

**Microscopic observations of cheeses (Gram stain or wet mount)**

- **Morphology:**
  - cheddar cheese
  - Limburger cheese

- **Gram reaction:**
  - Blue cheese (wet mount)

**Milk/Yogurt**

- **Lab 1**: initial pH: 
- **Lab 2**: final pH: 

**Odor of yogurt:**

**Consistency of yogurt:**

**Gram stain results:**

- Morphology: 
- Gram reaction: 

_TA Initials:_
EX. 18: Fungi – Results

Make drawings of the *Saccharomyces cerevisiae* cells. Magnification: 40/45X objective

![Saccharomyces cerevisiae](null)

For each of the molds, describe the following:

<table>
<thead>
<tr>
<th></th>
<th>Aspergillus</th>
<th>Penicillium</th>
<th>Rhizopus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dissecting Scope (agar plate)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draw or describe mold’s appearance:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Magnification:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light Microscope (prepared slide)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draw spore structure:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Magnification:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conidia or Sporangia?

**Two of the fungi have conidia.**

Septate or non-septate hyphae?

Color of colony **on agar plate**?

Generalized Structures for Molds:

- ![conidia](null)
- ![sporangium](null)
- ![non-septate/aseptate/coenocytic](null)
- ![septate](null)

TA Initials: