00

How To Keep a Laboratory Notebook

Introduction to the Notebook

Like sterile technique, microscopy, or biochemical analyses, keeping a current and accurate notebook is an essential skill in the microbiology laboratory. Your laboratory notebook will fulfill three primary functions in our course. First, it will give you the opportunity to develop a habit of properly documenting your laboratory activities. In a research lab, your notebook would be a permanent legal record of all your work. As such, it would be used to support grant applications, scientific publications, and patent applications by the members of your laboratory. Second, it will serve as your main study resource for the laboratory portion of our course. Your notebook should contain sufficient information to enable you or someone else to understand and reproduce the results of your work at a later date. This will be of particular importance as you review for our comprehensive laboratory practical exam at the end of this term. Finally, your notebook will help me to accurately evaluate your understanding of the laboratory materials and procedures that we use throughout the semester. By periodically collecting and grading your work, I can provide additional feedback to build upon your strengths and reinforce weaker areas so as to improve your learning this semester.

Supplies – This semester we will be using the hardcover *Student Laboratory Notebook* published by the American Society for Microbiology Press, 2005. (ISBN 1-55581-358-5) I have asked both the Ferris State University bookstore and Great Lakes Books to order copies for our labs. In addition, it can easily be found online at Amazon and other sites for about $12.00 (shipping is a couple dollars more). The style guide that we will be using in this class is *A Student Handbook for Writing in*

Biology, 4th edition by Karin Knisely, Sinauer Associates, 2013. (ISBN 978-1-4641-5076-0) This textbook will also be stocked by our bookstore and Great Lakes Books. Amazon and other online sites sell it for around $25.00. References to materials in Knisely's text will be abbreviated like this – *WIB* p 8. You are also going to need a solvent-resistant pen to write in your notebook. I find gel pens to be ideal for this purpose. The Pentel EnerGel with black ink is my personal favorite and can be found at our bookstores, Wal-Mart, Staples, and many other places for about $2.00 each.

Grading – Your completed laboratory notebook will be worth 60 out of the 1,200 total points this semester. I will collect all notebooks on two announced days during the semester (see your laboratory schedule for the exact dates). On each of these occasions, the accuracy, completeness, and format of your entries will be graded out of thirty possible points using the checklist provided in this document. I will also provide some feedback in your notebooks concerning the strengths and weaknesses exhibited in your entries. The point of this work, however, is not the grade. Rather, I am interested in helping you to create an accurate record of your activities and observations during the semester. In addition, completion of your laboratory notebook will give you an opportunity to apply the scientific method in the lab (*WIB* pp. 1-8).

General Instructions

Penmanship – Your laboratory notebook will be of little value to anyone if it is not legible and comprehensible when viewed at a later date. Begin each entry on an odd-numbered page (on the right side of the book). Write using a solvent-resistant ink pen. Write clearly, organize entries, include detailed drawings of observations when appropriate.

Personal information – It will be important for me to be able to identify each of your notebooks when grading. To that end – I ask that you label the cover and spine of your notebook as illustrated in Figure 1. This information should include your full name, course name, lab section number, and lab room number.

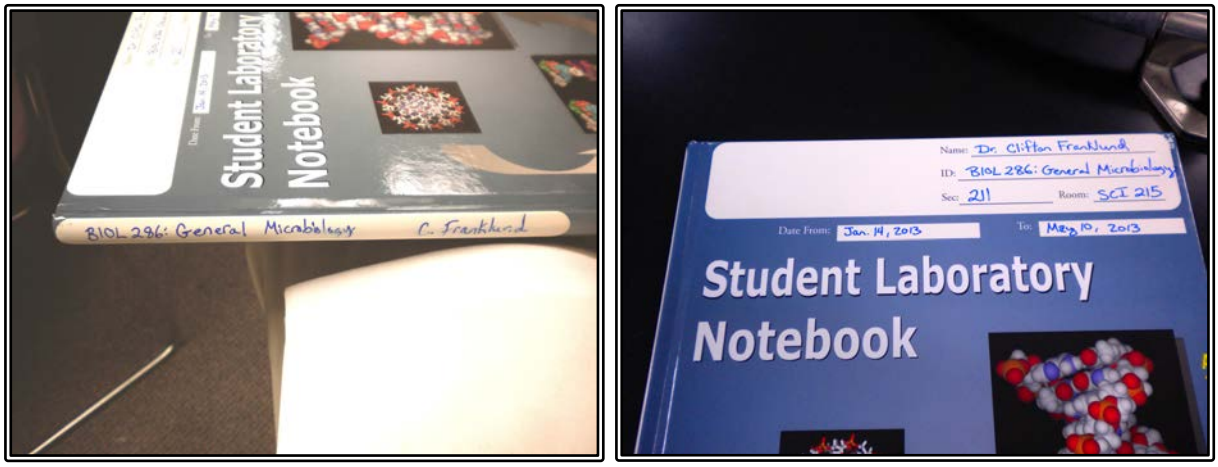

Figure 1 Properly labeled laboratory notebook spine and cover

Notebook checklist – You should print and cut out an extra copy of the laboratory notebook checklist found on our FerrisConnect site. Securely tape this copy onto the page facing the table of contents in your laboratory notebook. Refer to these guidelines when making your entries to ensure that they meet all of the notebook expectations when I am grading them.

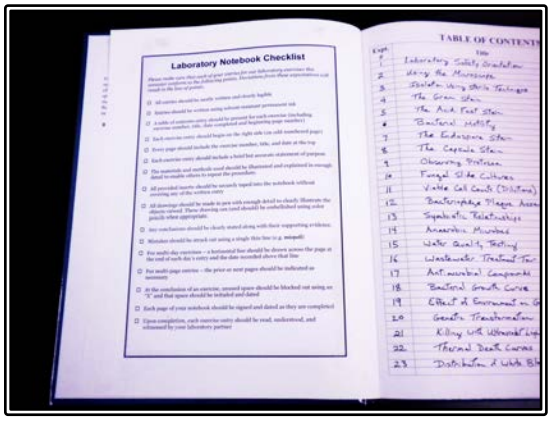

Figure 2 Properly placed checklist

Table of contents – Each laboratory exercise should have its own entry in the table of contents at the front of your notebook. Each entry should include the following information:

- The laboratory exercise number (in ascending numerical order)
- A brief but clear title (the same as on the top of each page for the entry)
- The date that work on the exercise began
- The first page number for the exercise entry

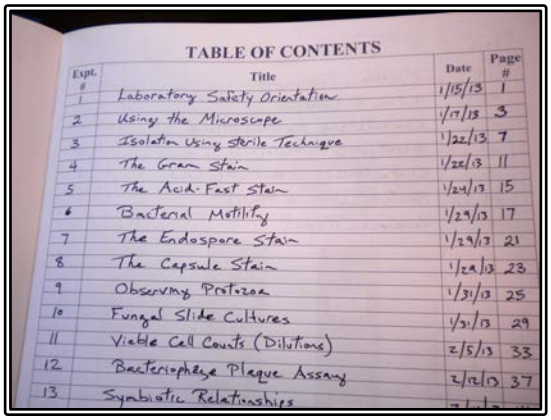

Figure 3 Proper table of contents

Multi-page entries – Most of our laboratory exercises will require more than one page to complete the entire entry. Fortunately, our notebooks provide an easy mechanism to facilitate longer entries. A the bottom of each leaf of your notebook, spaces are provided to indicate continuing pages. If you have filled in page 12 (for example) simply indicate "Continued on page 13" on the bottom of page 12 and "Continued from page 12" on page 13. You can (and should) use both sides of each notebook leaf. The only exception to this rule is that each new exercise should begin on an odd-numbered page. If this requires leaving the preceding page blank, you should mark it as described later in this document.

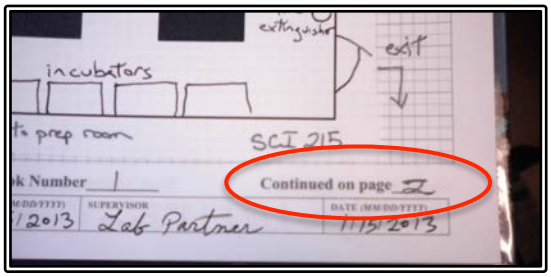

Figure 4 Continuing pages

Multi-day entries – Many of our exercises will require more than one day to complete. In these cases, you will need to indicate where each day's work in lab stopped and when you picked it back up again later. This is accomplished by drawing a horizontal line beneath the point where your current day's entry ends. You should sign and date your notebook above the line. Later, when you return to the exercise, you should indicate the date beneath the line and continue with the entry. Some of our laboratory exercises will span three or more class sessions and will require you to break your entries up into multiple sections in this manner.

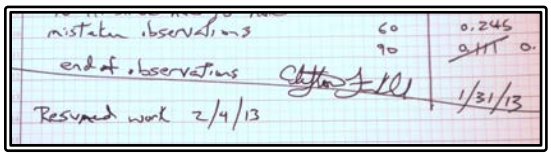

Figure 5 Multi-day entry

Correcting errors – Your laboratory notebook is a working document (like a journal) and mistakes *will* occur. The presence of errors in your entries will not result in a loss of points as long as they are properly corrected and they do not needlessly detract from the neatness, legibility, and clarity of your work. **NEVER** erase, blot out, or obscure information in

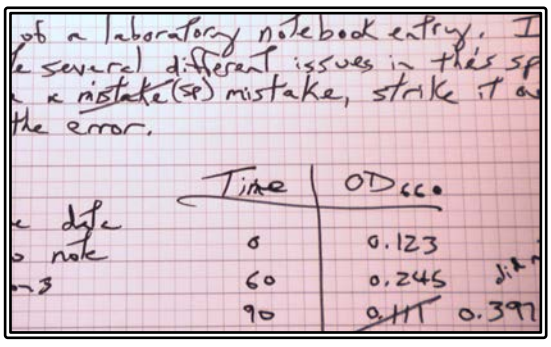

Figure 6 Correcting errors

your notebook. Neither should ever remove pages from your notebook. Instead, when you make a mistake in an entry simply strike through the material with a line (the entry should still be legible). You never know – what you thought was a mistake might turn out to be of some value later on during your work. It is also good practice to initial strikeouts and provide a brief explanation of the error (miscalculation, wrong sample name, etc.).

Inserting information – To simplify and standardize some portions of your laboratory entries, I will provide handouts to be included in your notebooks. You will cut these out to an appropriate size and permanently affix them to your notebook (scotch tape is preferred). Please indicate that there is nothing hiding beneath your insertions (and there should not be any overlapping materials in your entries). Loose sheets of paper in your notebooks are unacceptable.

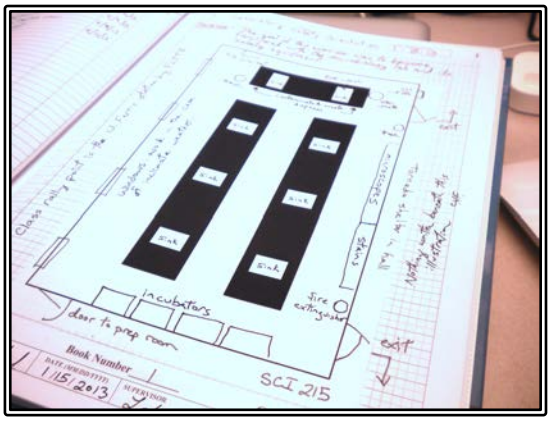

Figure 7 Proper notebook insertion

Unused space – Any unused space in your notebooks (whether a complete page or just a portion of a page) needs to be struck out and initialed. This is to demonstrate that additional data or observations were not added to your notebook at a later date.

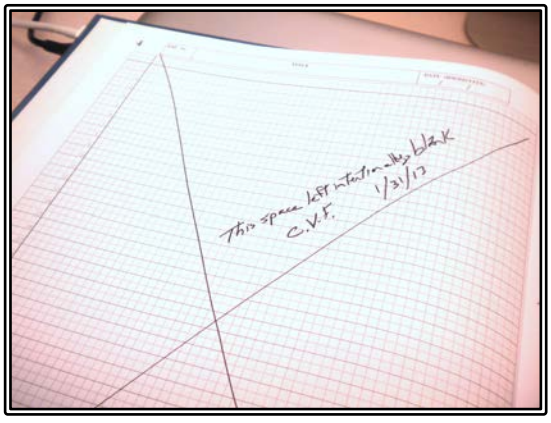

Figure 8 Marking unused space

Laboratory Entry Format

Your notebook entries should all have a common format and appearance. The six critical components of your entries are covered in out ancillary text. A brief description of each of these components is provided on the following page.

Title – (*WIB*, p 80) A short, but descriptive, title should be provided for each laboratory exercise. This title *must* appear at the top of each corresponding page in your notebook and also be present in the table of contents (in chronological order).

Purpose – (*WIB*, p 79) A very brief explanation of the activity's purpose should be present at the beginning of your entry. This only needs to be a few sentences long, but should adequately inform future readers about why you did the exercise and what you expected to find.

Procedure – (*WIB*, pp. 51-55) You should provide enough detail here to enable future readers to replicate your results. Alternatively, this section should have enough detail to remind you of what you did and how you did it when you need to reference it later this semester. You may cut and paste instructions from our lab handouts when applicable.

Observations – (*WIB*, pp. 55-75) Your observations in lab are amongst the most important parts of your notebook entries. Pay special attention to this part and put as much detail into your work as possible. Include tables of data, drawings, graphs, and other illustrations as you see fit. Please make sure to clearly label all illustrations, axes, and tables.

Conclusions – (*WIB*, pp. 75-78) Each laboratory exercise will include a few "critical thinking" questions. You will need to analyze your data and address these questions to complete the conclusion section.

Signatures and dates – One final point. You must sign and date every page of your notebook. In addition, you must have each page read and witnessed by a classmate. You can exchange notebooks with your lab partner to accomplish this.

Notebook Grading Rubric

Each of the 30 exercise entries in your laboratory notebook will be evaluated with the following holistic rubric. Your instructor will periodically ask to review you current work. Your entry scores will be recorded in your Table of Contents. Lab notebook scores will be released online (in FerrisConnect) in two 30-point batches. Neglecting to stay current in your notebooks will cost you points! So, keep on top of these resources.

+	**Excellent** *(2.0 points)*	The notebook entry contains all required components (title, date, purpose, procedure, observations, conclusions, and Table of Contents entry). Each component is complete, accurate, and completed in pen. The student has signed and dated each page of the entry. A few minor (and easily correctible) mistakes may be present in the entry.
✓	**Adequate** *(1.5 points)*	The notebook entry contains all required components (title, date, purpose, procedure, observations, conclusions, and Table of Contents entry). Most components are complete, accurate, and completed in pen. The student has signed and dated most pages of the entry. A few factual mistakes may be present and the observations may lack sufficient detail.
–	**Needs Improvement** *(1.0 point)*	The notebook entry is missing one of the required components (title, date, purpose, procedure, observations, conclusions, and Table of Contents entry). Most components are complete, accurate, and completed in pen. The student has signed and dated most pages of the entry. A few factual mistakes may be present and the observations may lack sufficient detail.
0	**Poor** *(0.0 points)*	The notebook entry is missing two or more of the required components (title, date, purpose, procedure, observations, conclusions, and Table of Contents entry). Few of the components are complete, accurate, and completed in pen. The student failed to sign and date most pages of the entry. Many factual mistakes may be present and most of the observations lack sufficient detail.

Laboratory Notebook Checklist

Please make sure that each of your entries for our laboratory exercises this semester conform to the following points. Deviations from these expectations will result in the loss of points.

- ☐ All entries should be neatly written and clearly legible
- ☐ Entries should be written using solvent-resistant permanent ink
- ☐ A table of contents entry should be present for each exercise (including exercise number, title, date work began, and beginning page number)
- ☐ Each exercise entry should begin on the right side (an odd-numbered page)
- ☐ Every page should include the exercise number, title, and date at the top
- ☐ Each exercise entry should include a brief but accurate statement of purpose
- ☐ The materials and methods used should be illustrated and explained in enough detail to enable others to repeat the procedure.
- ☐ All provided inserts should be securely taped into the notebook without covering any of the written entry
- ☐ All drawings should be made in pen with enough detail to clearly illustrate the objects viewed. These drawing can (and should) be embellished using color pencils when appropriate.
- ☐ Any conclusions should be clearly stated along with their supporting evidence.
- ☐ Mistakes should be struck out using a single thin line (e.g. ~~mispell~~)
- ☐ For multi-day exercises – a horizontal line should be drawn across the page at the end of each day's entry and the date recorded above that line
- ☐ For multi-page entries – the prior or next pages should be indicated as necessary
- ☐ At the conclusion of an exercise, unused space should be blocked out using an "X" and that space should be initialed and dated
- ☐ Each page of your notebook should be signed and dated as they are completed
- ☐ Upon completion, each exercise entry should be read, understood, and witnessed by your laboratory partner

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Laboratory Safety

Background

Right to Know / Chemical Labeling - You have a right to know (and are expected to know) about all chemicals (including consumer products) you will be exposed to in the lab class. All materials will be labeled with the NAME (no abbreviations) of the material and its HAZARD if any (e.g. flammable, corrosive, oxidizer etc.).

Material Safety Data Sheets (MSDS) - Additional information about chemicals/products is available from the Ferris Environmental Health and Safety officer in the form of Material Safety Data Sheets. Requests for MSDS will be honored. I suggest that you search for the appropriate information on-line at http://www.msdssearch.com. Use the DB tab to search several web databases.

Laboratory Attire

Clothing - The laboratory is a dirty and potentially dangerous place. To minimize the likelihood of personal injury you should avoid loose-fitting clothing. I also strongly recommend that you wear older clothes in the lab – the stains that we use bind very avidly to cloth.

Hair - If your hair is long enough to be tied back, please do so in the laboratory. The smell of singed hair in the Bunsen burners is most unpleasant.

Shoes - Close-toed footwear must be worn at all times in the lab. Your shoes should be appropriate for the lab; avoid high heels!

Lab coat - You are required to purchase and wear a lab coat during all laboratory sessions. The coat will protect you and your clothing from biological contamination and chemical spills. I may allow a non-standard garment in place of a lab coat provided that it is of an appropriate size and composition.

Goggles - Safety goggles must be worn when working with hazardous materials or conditions that can injure the eyes or skin. You will be instructed to don your goggles when required to do so.

General Policies

Attendance - Because the laboratory is an essential component of this course, I will be keeping attendance. Students with more than two unexcused absences will fail the course.

Conduct - Inappropriate behavior will not be tolerated. All members of the class - students and instructor - will treat each other with respect. I have the right to ask a student to leave if he or she is behaving inappropriately, disrupting class, or refuses to promptly and fully comply with any of these safety policies.

No eating/drinking/food/smoking - No food or drink is allowed in the laboratory. Students must leave these things outside, or keep them in their closed backpacks. THIS INCLUDES PERSONAL WATER BOTTLES!

Reporting Accidents/Incidents - Injuries, dangerous equipment failures, chemical spills, etc. must be immediately reported to me. Injured students should go to the Student Health Center for medical evaluation. You may walk there with an escort or may be transported by University Police. Do not attempt to clean up blood other than your own!

Health concerns - If you are currently taking any medications that suppress your immune system, know yourself to have any condition that compromises your immune system, or are pregnant (or have reason to believe you might be), you must notify your instructor. In most cases you will be able to participate in all the experiments; however,

this information will ensure that any additional precautions that might be required for your safety are implemented. To maintain the confidentiality of this information any such additional precautions will be applied to all students.

Bench cleaning - At the beginning and end of each lab session you should wipe your workspace down with an antiseptic (Roccal). Please dispose of any dirty paper towels in the regular trash bins.

Handwashing - The single most important procedure to protect you this semester is regular and thorough handwashing. At the end of each lab session (and anytime that you believe that you may have contaminated yourself) you should wash your hands using the provided antimicrobial soap and hot water.

Spills - If any water is spilled on the floor, it must be cleaned up immediately to prevent slip/fall injuries or damage to the rooms below and adjacent to the lab. If the water can't be easily cleaned up, notify me so that I can get a spill kit.

Waste Disposal

Housekeeping - You must clean up after yourself. Trash must not be left in the room, thrown into drawers, sinks, on the floor, *etc*. Sinks clogged with student trash can cause a flood in the lab.

Regular trash - Please put all of your non-contaminated trash in the regular trash bins. DO NOT PUT SOLID TRASH IN THE SINKS! This includes used matches.

Stains - Do not dump stains down the drain. There is a large labeled carboy at the front of the lab for stain waste collection.

Glass - Broken glass should be reported to me – do not attempt to clean it up yourself. Glass waste is collected in a separate container. Please do not put glass waste in the regular trash – our custodial staff thank you.

Chemicals - I will provide containers and instruction for disposal of chemical wastes generated in the lab. Do not dump anything into the sinks unless I have specifically instructed you to do so.

Contaminated materials - Small spills should be saturated with Roccal and wiped up with a paper towel. Place these towels in the orange autoclave bag at the front of the lab. When you are done with cultures, they should be disposed of in the orange autoclave bag. Contaminated glassware will be collected in a bin at the front of the lab for later decontamination.

Safety Equipment

Eyewash - There is an emergency eye wash in the sink at the front of the lab. The path to this wash must remain clear during class. The shower stays ON when the handle is pulled. You must use the wash for 15 minutes minimum. During the 15-minute flushing, you should use your fingers to hold your eyelids open. Do not worry about looking silly or making a mess – your vision is at stake!

Fire alarm - In case of a fire there is an alarm pull in the hallway outside of our laboratory. If appropriate, pull the alarm and evacuate the building.

Fire extinguisher - An A/B/C class fire extinguisher is mounted on the wall near the back of the lab. Trained people should only use this device. DON'T BE A HERO. Evacuate the room and pull the fire alarm as appropriate.

Fire blanket - There is also a fire blanket mounted at the front of the lab. To use, open the fire blanket and hold it in front of you to shield your body (especially the face and hands) from the fire. Cover the burning material completely, ensuring there are no gaps for oxygen to reach the fire. Leave the blanket in place, allowing the material to cool for at least 30 minutes before lifting the blanket. If appropriate, shut of the gas or electrical supply if it is involved in the fire. Contact the fire department if you have not already done so.

Evacuation Route - If the fire alarm sounds, or you are ordered out of the lab, you must immediately leave the building taking your backpacks with you! If possible, turn off Bunsen burners and other equipment before leaving. I will tell you the safest and quickest route out of the building. EVERYONE MUST GET 200 FEET AWAY from the building – do not bunch-up just outside the doorway!

Tornado policy - If a tornado warning is announced during class, we will move to a designated storm shelter - in our case, the hallway outside of the lab. Keep all doors shut and avoid windows as much as possible. If you are unable to get out of the lab in time, take cover beneath our rather substantial laboratory benches.

Laboratory Hazards

Bunsen burners - You must follow my instructions for lighting and using Bunsen burners in the lab. Never EVER leave a lit Bunsen burner unattended – the blue flame is difficult to see in a well-lit lab setting.

Bacterial stocks - Most of the experiments you will do in the microbiology lab involve the use of living microbes. Although many of these microbes are considered non-pathogenic, not likely to cause disease in healthy individuals, they must all be handled as though they were pathogenic, likely to cause disease in healthy individuals. Safe handling of microorganisms will be emphasized as you perform each of the experiments, both to protect you and your classmates from infection and to ensure that the microbes themselves do not become contaminated with other unwanted microbes. The organisms that we use in our laboratory exercises are classified as either Biosafety Level 1 (BSL-1) or Biosafety Level 2 (BSL-2).

> **BSL-1**: These microbes include well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. Typical examples of BSL-1 organisms include non-pathogenic strains of *Escherichia coli*, *Bacillus cereus*, and *Saccharomyces cerevisiae*.

BSL-2: These microbes there pose a moderate hazard to people and the environment. They are typically indigenous and associated with diseases of varying severity. However, infections are not easily transmissible and effective treatments are readily available. An example of a microbe that is typically worked with at a BSL-2 laboratory is *Staphylococcus aureus*.

Other - Specific instructions will be provided when you are working with other potentially chemicals or conditions. These may include ultraviolet radiation, combustible gases or solutions, caustic chemicals, or high-voltage equipment. Stay alert and follow instructions.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exerci se are given below.

✪ Observations

After reviewing our safety guidelines, you should identify and illustrate to locations of the following objects:

1) All windows and doors in our lab.
2) All lab benches and the location of your seat in the lab.
3) The emergency gas shut off valve.
4) The fire extinguisher
5) The fire blanket
6) The eyewash station
7) The stain waste barrel
8) The contaminated waste buckets
9) The broken glass boxes
10) The regular trash cans

You can illustrate all of this with a simple floor plan sketch or map.

✪ Conclusions

- What is your laboratory drawer number?
- What is you microscope number?
- Where is the tornado evacuation location?
- What is the most dangerous piece of equipment in our lab?
- What biosafety level are most of our bacterial stocks in the lab?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

There should be no glass waste today. However, you should now know where to dispose of broken glass in the lab.

Compound light microscope

Although we did not yet use the microscopes in lab, you should have signed up for a specific instrument. Make sure that you can locate it in lab.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

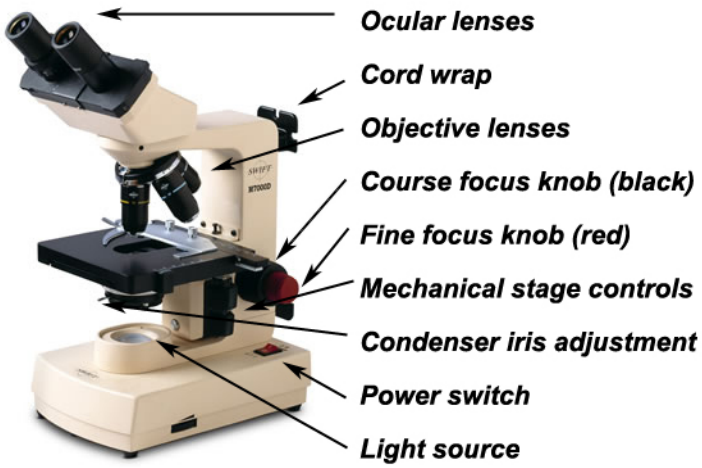
You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

02

Microscopy

Background

We are using Swift M7000 binocular compound light microscopes in our microbiology laboratories. This document pertains to the proper use of our particular microscopes. You are responsible for all of the material covered in both this document and any other reading assigned by your laboratory instructor.



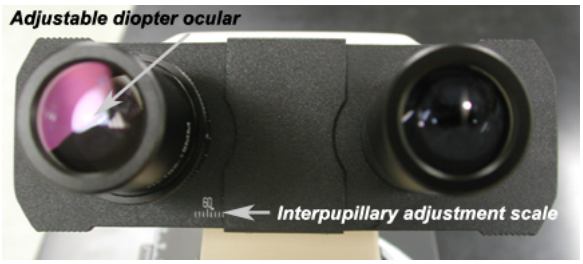
Major parts of the M7000 microscope:

The essential parts of our microscopes are labeled to the left. All compound microscopes share most of these features. Note that our microscopes have two 10X ocular lenses (binocular). This make viewing more comfortable, but requires a few extra adjustments to be fully appreciated. The function of each component will be described below:

- **Ocular lenses**: The eye pieces – each has a magnifying power of 10X.
- **Cord wrap**: Brackets to store the power cord around when done using the scope.
- **Objective lenses**: Secondary lenses to magnify the sample – these range from 4X to 100X.
- **Course focus knob**: Used to rapidly move the stage (an sample) to focus.
- **Fine focus knob**: Used to tweak the focus – use this with the 40X and 100X objectives.
- **Mechanical stage controls**: Move the sample in the X and Y planes. Focus is the Z plane.
- **Condenser iris**: This lever controls how much light passes through your sample.
- **Power switch**: Turns the lamp on and off.
- **Light source**: A halogen lamp.

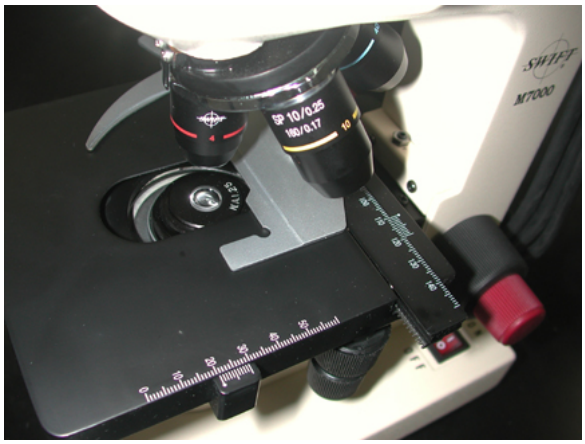
Procedure for taking out and using the microscope:

1. Go to the microscope cabinet and get the scope that was assigned to you. Carry it carefully back to your bench using two hands (one holding the arm of the scope and one under the base).
2. Unwrap the power cord and plug in the microscope.
3. Make sure that the 4X objective is pointed down and that the stage is as far away from the objective as possible.
4. Turn on the lamp.
5. Adjust the condenser iris lever to regulate the degree of illumination.
6. Set the interpupillary distance between the ocular lenses. When set correctly, you will comfortably see a single circular field of view as you look through the eyepieces. This is accomplished by gently pushing the ocular lenses together or apart while viewing through them. When you get them right, note the setting on the adjustment scale. The next time that you use a microscope, you can quickly set up the interpupillary distance using your prior measurement.



7. Place the slide with your specimen (right side up!) onto the stage and carefully secure with the spring-loaded slide holder.
8. Use the mechanical stage controls to center your specimen in the illuminated area over the condenser.
9. Double-check that the 4X objective is pointed down toward your sample.
10. While viewing through the ocular lenses, use the coarse focus knobs (black) to quickly bring the slide into focus. Adjust the degree of illumination using the condenser iris lever.
11. Now close your left eye. While viewing with your right eye, use the fine focus knobs (red) to bring the specimen into sharp focus. You should see the ocular reticle (measuring scale) in the right eyepiece.
12. Next, close your right eye. While viewing with your left eye, use the diopter adjustment to bring the specimen into sharp focus. Rotating the left ocular lens does this. The diopter adjustment makes allowances for the fact that your vision in your left and right eyes is probably not the same.

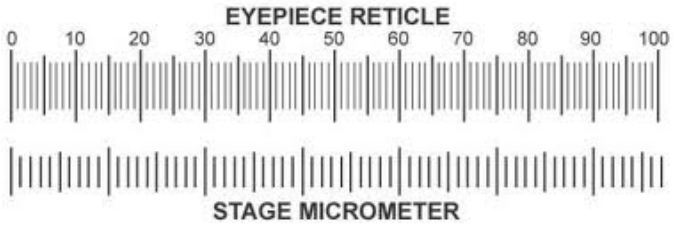
Now you are ready to use the other objective lenses. Our microscopes have four different objectives lenses. These allow you to view objects at a variety of different magnifications. Our microscope objectives are **parfocal** and **parcentered**. This means that once the sample is centered and focused with one objective, it will be (more or less) focused and centered for all of the other objectives too. Please note that the **total magnification** achieved is the product of the ocular lens (10X) and the objective lens. As magnification increases, the **field of view** (total area of the sample that you can see) decreases. But even more important is the fact that as the magnification increases the **working distance** (space between the objective lens and the sample) decreases. *For this reason, you should use only the fine focus to adjust your microscope from this point on*.



13. Rotate the objective turret so that the 10X objective points toward your sample and adjust the focus using the fine focus knobs (red). You may use the mechanical stage knobs to re-center your specimen in the field of view. Note that the microscope inverts the image (? appears as ¿ – if you don't believe me, use try moving your mechanical stage while viewing the letter e slide). As a consequence, when you move the stage left, the image appears to move right. This takes some getting used to.
14. Now rotate the objective turret so that the 40X objective points toward your sample and adjust the focus using the fine focus knobs (red). You may use the mechanical stage knobs to re-center your specimen in the field of view. This is enough magnification to begin to see the shape and arrangement of prokaryotic cells.
15. Finally, rotate the objective turret so that the 100X objective points toward your sample. You will need to place a drop of immersion oil onto your slide to use this objective. *Do not get oil on the other objective lenses!* Then adjust the focus using the fine focus knobs (red). You may use the mechanical stage knobs to re-center your specimen in the field of view. This is the highest magnification that we can achieve in our labs – you should be able to clearly see the size and arrangement of bacterial cells.

Calibrating and making measurements using the microscope:

Your right ocular lens should have a measuring reticle (scale) with 100 unit-less divisions. These marks can be used to estimate the size of microbes and other structures. However, in order to accurately measure objects, we first need to calibrate – standardize – the reticle divisions. To this, we will use a special slide with a stage micrometer. These slides have a line etched on them that is **1 mm (1,000 µm)** long and divided into 100 equal segments (**10 µm each**). You will focus on this line using each of your objective lenses in turn. By aligning the scales, you can determine how many ocular divisions correspond to how many slide divisions. For example: in the image to the right, 30 ocular divisions spans 2o divisions on the stage micrometer scale. Since each stage micrometer division is 10 µm, that makes 20 X 10 µm = 200 µm. This is then divided by the number of ocular divisions 200 µm ÷ 30 = 6.7 µm per division. Now if you use this object lens and observe a diatom to be 12 divisions long, you can estimate its length to be 12 X 6.7 = about 80 µm. The apparent size of each division will vary as you adjust the total magnification of the microscope.



Procedure for putting away the microscope:

When you have finished observing with your microscope, complete these steps before putting it back into the cabinet.

1. Rotate the objective nosepiece so that the 4X objective points toward your stage.
2. Remove your slide (if one is present) from the mechanical stage.
3. Carefully wipe any immersion oil off of the 100X objective *using lens paper* (ONLY!).
4. Use the coarse focus knobs (black) to lower the stage as much as possible.
5. Turn off the power to the substage lamp.
6. Unplug the microscope and wrap the electric cord up in the provided location on the microscope arm assembly.
7. Carry the microscope carefully back to the cabinet using two hands (one holding the arm of the scope and one under the base).
8. Place your microscope back into the correct cubby.

Immersion oil may be removed from commercially prepared microscope slides using Kimwipes. These slides should be returned to where you found them.

Any microscope slides that you have prepared in lab can be disposed of in the glass waste box.

DO NOT put the microscope away with oil on the lenses or slides still on the stage! I will give a warning for the first improper use and storage of our microscopes. Thereafter, I will deduct laboratory points.

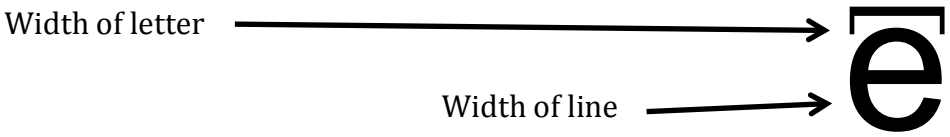
Instructions

Focus on the stage micrometer with each of your microscopes objective lenses. Align the ocular reticle with the stage micrometer and illustrate the relative spacing. Using this information, **determine the relative size of each ocular reticle division** (in µm) for your microscope.

The entire ocular reticle (all 100 divisions) spans 56% of the field of view. Using this information, **calculate the total field of view for each of your objective lenses**. Also, use a ruler to **measure the working distance for each objective** (this is the distance between your objective lens and the surface of the slide when in focus).

Use an appropriate calibrated objective lens to measure the width of the letter "e" on the prepared slide.

Also estimate the width of the line making up the letter. Draw what you see…



Notebook Entry

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✪ Observations

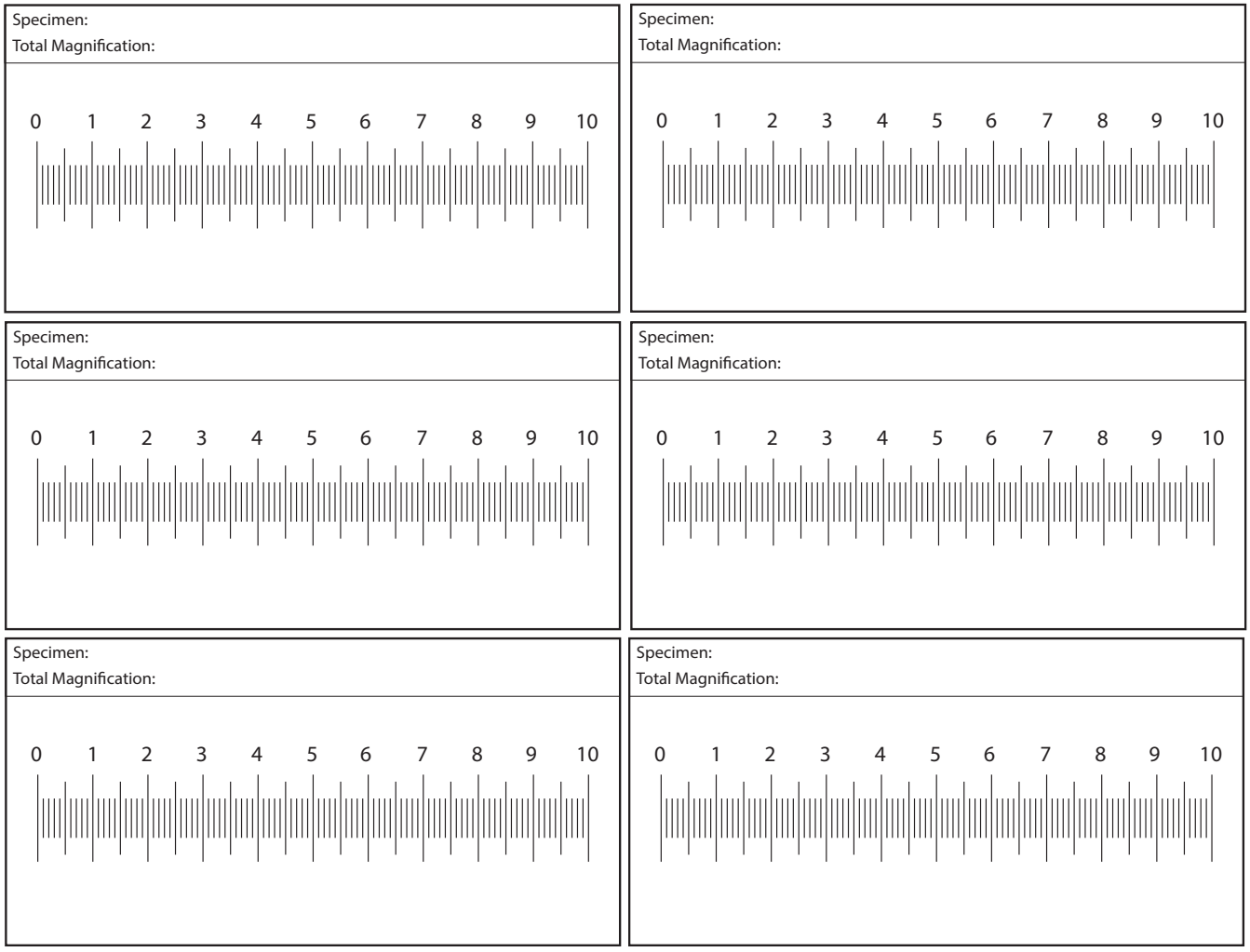
• Record the view of the stage micrometer and ocular micrometers in focus for each of your objectives.

• Record the view of the letter e slide at an appropriate magnification.

You may record these data in one of two ways:

1) Photograph the view through your right ocular with your digital camera, print the picture, tape it into your lab notebook and label it appropriately.

2) Using the panels below, draw a black bar beneath the ocular scale and indicate how many stage division it represents. For example, if 12 stage divisions equaled 40 ocular divisions, the black bar should extend from 0 to 4 and be labeled "12". Draw the letter e to scale against the ocular divisions.



Calculate the size of an ocular division at each magnification and the dimensions of the letter e (show your calculations).

✪ Conclusions

Answer the following questions and complete this data table.

1) How did the letter “e” appear when you look directly at the slide? How did it appear when viewed through your microscope?
2) How wide was the letter “e” (in µm)?
3) How wide is the letter “e” line (in µm)?

Objective	Magnification	Working Distance	Field of View	Division Size
4X				
10X				
40X				
100X				

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

There should be no glass waste today. However, you should now know where to dispose of broken glass in the lab.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope’s arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Commercial Slides

Do not throw the commercial slides away! Wipe any immersion oil off of the slides using a Kimwipe. Then return the slide to the correct slide box.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

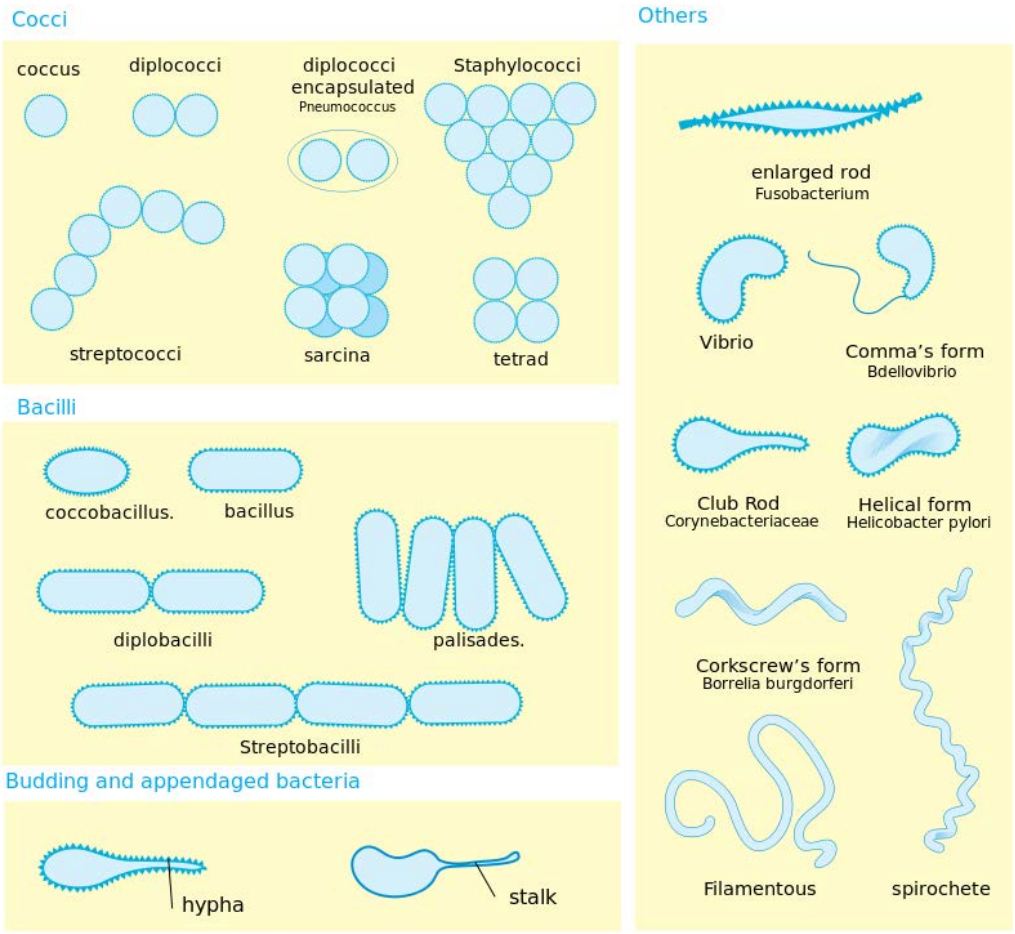
You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

03

Bacterial Cell Morphology

Background

Being able to locate, observe, and record bacterial cells using our compound light microscopes is an essential laboratory skill. In today's exercise, you will use commercially prepared slides containing three different bacterial strains. These bacterial cells represent the three most common shapes – cocci (balls), bacilli (rods), and spiral (curved rods). Some of the most common shapes and arrangements of bacterial cells are illustrated below. You will need to be conversant with this term for both our lecture and laboratory assignments.



Instructions

Each student should get one prepared microscope slide (there will be three distinct patches of stained bacteria on slide). Center and focus the slide using a low-power objective (4X or 10X) first. Then switch to high dry (40X) and focus using the fine focus – red knob – only. Finally, rotate the 40X objective out of the way and place one drop of immersion oil on the slide (where it its being illuminated). Rotate the 100X oil immersion lens into the drop of oil and focus using fine focus only. You should photograph or draw the cells that you see to scale. Use proper terminology to describe their shape and arrangement and give an estimate of their size in μm based upon your calibrated micrometer.

Notebook Entry

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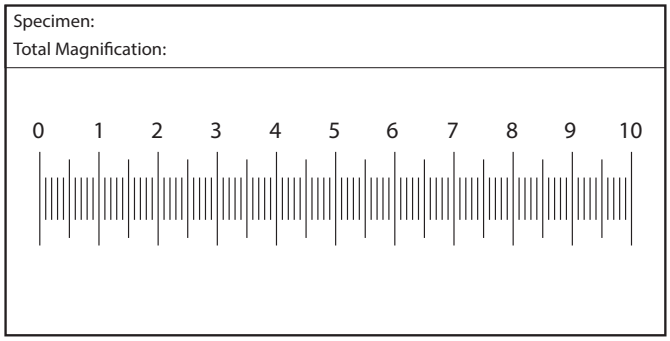
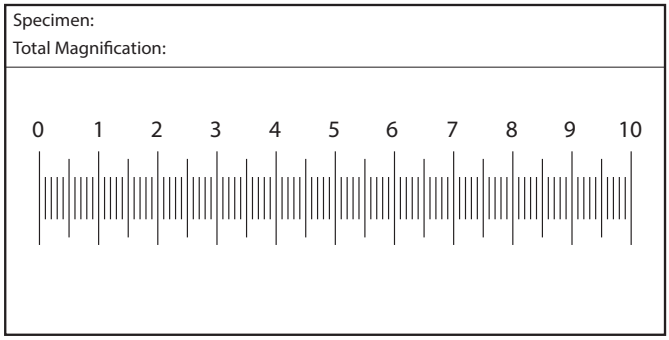
✪ Observations

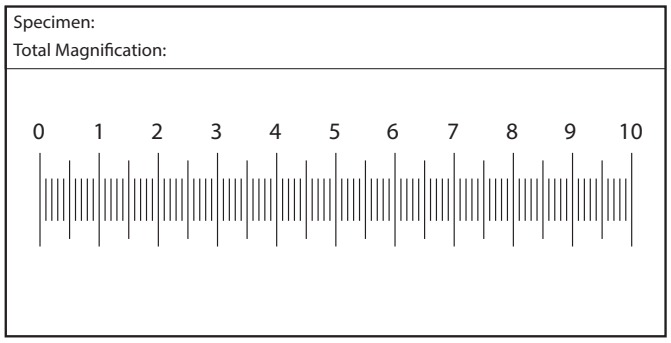
• Record the view of the ocular micrometers and bacterial cells in focus for each of the three types of microbial cell shapes.

You may record these data in one of two ways:

1) Photograph the view through your right ocular with your digital camera, print the picture, tape it into your lab notebook and label it appropriately.
2) Using the panels below, draw a black bar beneath the ocular scale and indicate how many stage division it represents. For example, if 12 stage divisions equaled 40 ocular divisions, the black bar should extend from 0 to 4 and be labeled "12". Draw the letter e to scale against the ocular divisions.







Calculate the size of each type of microbial cell (show your calculations).

✪ Conclusions

Answer the following questions and complete this data table.

1) What are the three shapes of cells that you saw? (use the terms from the figure)
2) Which of the cells was the longest?
3) Which of the cells was the smallest?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

There should be no glass waste today. However, you should now know where to dispose of broken glass in the lab.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Commercial Slides

Do not throw the commercial slides away! Wipe any immersion oil off of the slides using a Kimwipe. Then return the slide to the correct slide box.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

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04

Aseptic Technique

Background

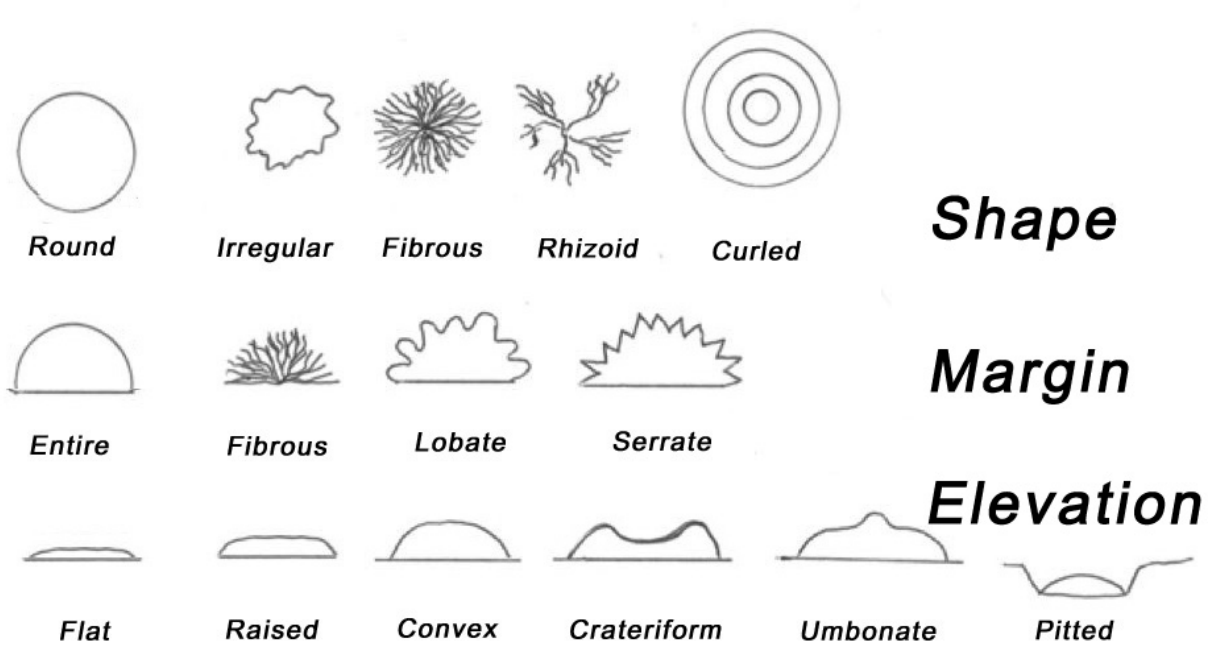
Accurate characterization and identification of microbes relies upon access to pure cultures. Unfortunately, microbial cultures are nearly always complex mixtures in nature. During the "Golden Age" of microbiology (19th century), many different groups worked to find efficient means to isolate single microbes from these mixed communities. In Koch's laboratory, agar was used to make irreversible solid media for the first time. This allowed microbiologists to easily isolate bacteria by streaking. Streaking for isolation is a form of dilution on a solid surface. use and storage of our microscopes. You will be practicing this technique today with a simple mixed culture of bacteria.

When we grow pure or mixed cultures on solid media, microbes appear in large enough numbers to be visible to the naked eye. These colonies of microbes can be distinguished by several different physical characteristics. Below is a partial listing of some more common morphological features used to describe colonies in the lab.

Size - Microbial colonies can vary quite a bit in their overall size (diameter). Punctiform colonies appear as pinpoints. Most other colonies have diameters that can be measured in mm.

Shape - The overall shape of the colonies can vary as well. Some of the common shapes are illustrated below, and include the following: **Round** - many colonies have a nice radial symmetry and appear as round mounds of growth. **Irregular** - other colonies have less symmetry and have undulating edges. **Fibrous** - some soil bacteria have fibrous or hairy colonies. **Rhizoid** – a few bacteria (and many fungi) have a spreading and branching type of growth. **Curled** - due to swarming on the plate, some colonies have a terraced appearance that is called curled.

Margin - The edge, or margin, of the colony may also have several appearances as drawn below. **Entire** - colonies with a smooth and even edge are referred to as entire. **Fibrous** - some colonies have a filamentous edge and are called fibrous. **Lobate** - other colonies can



have an irregular, lobed edge. **Serrate** - finally, some can have a saw-tooth edge which is called either **serrate** or **erose** (eroded).

Elevation - The height, or elevation, of the colony may also have several appearances as drawn above. **Flat** - some colonies have very little elevation and remain flat on the agar surface. **Raised** - many colonies have some elevation and appear somewhat raised from the surface. **Convex** - other colonies form a substantial pile of cells with a dome-like structure. **Cateriform** - a few colonies exhibit depressions in the center and could also be called concave. **Umbonate** - still others have an extra dimple in the center. **Pitted** - rarely, some microbes are able to metabolize the agar in the media. This can result in the colonies pitting into the growth medium.

Color - Most microbial colonies have similar coloration; there are lots of variants of cream to tan. However, there are examples of microbes that produce white, black, yellow, red, pink, purple, and other colored colonies.

Consistency - The overall consistency of the microbial culture can be tested by touching with a sterile loop. Some of the common consistencies include: **Butyrous**; Most bacterial colonies have a buttery consistency. **Viscous**; Others are more sticky. **Mucous**; Still others have a slimy consistency. This is often due to the production of a capsule. Friable. Some colonies (like the mycobacteria) have hard and waxy colonies that break up upon probing.

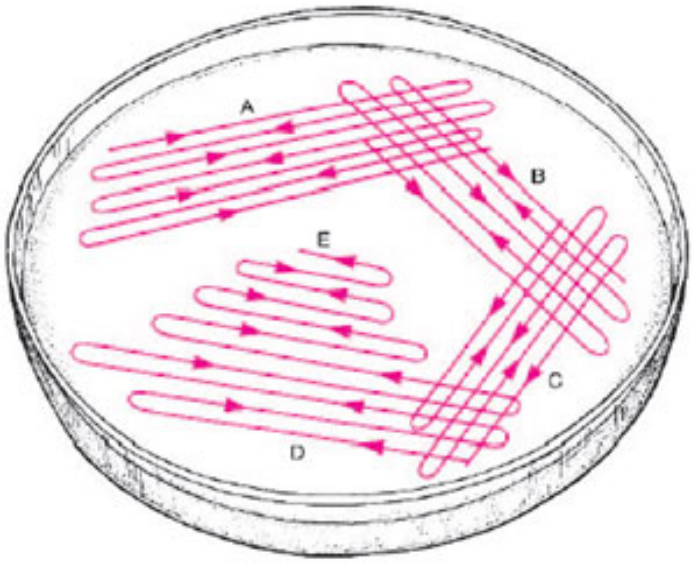
Surface - The surface of the colonies can also vary. Most appear to be smooth and/or shiny. The presence of a capsule can contribute to this. Others appear dull or rough, while others yet may be wrinkled.

Opacity - Lastly, the colonies can vary in their opacity. Most will appear to be opaque on the plates. However, others will have varying degree of translucence.

You should note that colony morphology varies from bacterium to bacterium. However, the morphology is also strongly affected by the growth medium and environmental conditions (temperature, humidity, etc) used. In general, we assume that a pure culture will only exhibit one kind of colony morphology, while a mixed culture should have more than one type of colony present. As always, there are exceptions to this rule.

Instructions

You will be given one plate of media and a mixed culture containing *Escherichia coli* and *Staphylococcus aureus.* Sterilize a wire loop by incineration and let it cool for about 10 seconds. Then get one loopful of the mixed culture and apply it to your plate as shown in "A" to the right. Sterilize your loop again and let it cool. Now use one edge of your loop to spread as shown in "B". Turn you loop around and use the other edge to spread as shown in "C". Finally, sterilize your loop one last time and let it cool. Complete the streaking as shown in "D" (use the rest of the available area). Label the bottom of your plate with your name, lab section, date, and sample information using a Sharpie. Put your plate in the 35°C incubator on your lab section's shelf (bottoms up).



After 24 to 48 hours, the microbes will have multiplied enough that macroscopic piles of microscopic cells (colonies) will be visible on your plates. If you have succeeded, some of these colonies will be isolated – not touching other colonies. We make the assumption that such colonies arose from one isolated cell. Since bacteria reproduce asexually by binary fission, all of the cells in a colony should be genetically identical (the colony should be clonal).

Notebook Entry

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✪ Observations

• Record the view of your streak plate after incubation.

You may record these data in one of two ways:

1) Photograph the plate with your digital camera, print the picture, tape it into your lab notebook and label it appropriately. Make sure that the colony morphology is discernable.
2) Draw the appearance of your streak plate by hand. Make sure that degree of isolation and the colony morphology is discernable.

✪ Conclusions

1) Did you see isolated colonies?
2) How many colony types did you see?
3) Describe the morphology of your colonies.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

There should be no glass waste today. However, you should now know where to dispose of broken glass in the lab.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. We are using a mixed culture of:

1. *Escherichia coli* (BSL-1). This is a common member of your gut microbiota.
2. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed streak plate.

Contaminated materials

When you have completed your observations of the petri dish (following incubation), you should dispose of the plates in the lined silver buckets at the front of the laboratory.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

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05

Gram Stain

Background

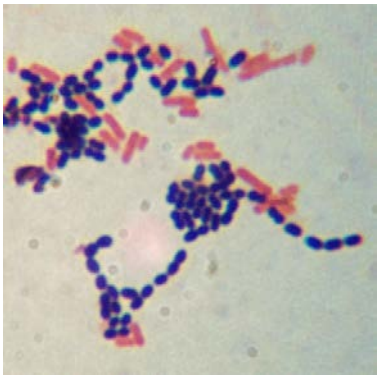
The Gram stain was devised by a Danish physician, Hans Christian Gram, while he was working in Berlin in 1883. At the time, Dr. Gram was studying lung tissue sections from patients who had died of pneumonia. He was frustrated by the fact that common tissue staining procedures were inadequate to differentiate between host cells and microbial pathogens. So he set out to devise a more efficacious stain. Through a process of experimentation, Dr. Gram discovered that Gentian (crystal) violet and a solution of iodine mixed with potassium iodide formed a water-insoluble precipitate. This precipitate could be removed from host tissues and some microbial cells by an alcohol rinse. Many other microbes, however, retained the stain complex. This stain quickly became one of the main techniques used to initially characterize bacterial isolates. Like all differential stains, the Gram stain consists of four steps. These are summarized in the table below.

Step	Compound	Time	Gram Positive	Gram Negative
Primary Stain	Crystal Violet	1 minute	Purple	Purple
Mordant	Gram's Iodine	1 minute	Purple	Purple
Decolorizer	Acetone Alcohol	~ 5 seconds	Purple	Colorless
Secondary Stain	Safranin	1 minute	**Purple**	**Pink**

For over 100 years, the Gram stain reaction has been thought to be associated with the cellular architecture of the bacterial cell wall. However, the actual mechanism of this stain was only worked out relatively recently. During staining, crystal violet-iodine complexes form inside the bacterial cells and on the cell walls. The decolorizing agent (acetone alcohol) dehydrates the cells and causes some leakage across the cell membrane. The intracellular dye complexes are trapped within gram positive cells due to the dense peptidoglycan saculus following dehydration. In gram negatives, however, the outer membrane is dissolved by the decolorizer and the peptidoglycan layer is insufficient to retain the dye complexes. Hence, the gram negative cells lose their color. The counterstain allows these cells to be visualized. The mechanism involved in this process is illustrated in my little video below.

Although widely used, the Gram stain does not work well for all microbes. Spirochetes like *Borellia*, *Treponema*, and *Leptospira* are too thin to be resolved by brightfield microscopy - even when stained. In addition, some microbes are refractory to staining. The acid-fast bacteria (*Mycobacterium*) were recognized as difficult to stain by even Dr. Gram. This is due to the difficulty in getting stains into these microbial cells due to the waxy mycolic acid layer in their cell wall. Other intracellular bacterial parasites, like *Bartonella*, *Chlamydia*, and *Rickettsia*, are also known to be difficult to stain with analine dyes (such as crystal violet).

To complicate things further, even cells that are properly stained by this procedure can give variable results. One common reason for staining variability is the age of the culture. Many bacterial cells become more fragile with age (eg in stationary and death phase) and destain much more easily than younger cells. Other gram positive cells have a less substantial peptidoglycan layer and are, as a consequence, easier to destain. Confusion can arise when trying to describe the organisms in this section.



A proper Gram stain should result in either dark purple gram positive bacteria or lighter pink gram negative cells. A good stain from a mixed culture is shown to the right. This culture consisted of gram negative bacilli (primarily present as individual cells) and gram positive streptococci (spherical bacteria mostly found in chains of four or more cells). To give you and idea of the scale involved - the cocci in this image are a little under 1 μm in diameter while the bacilli are about 1 μm wide and 3 μm long. Due to its speed, low cost, and utility, the Gram stain is usually the first test performed when identifying a clinical isolate. In this exercise, you will be practicing the

Gram stain on eight different mixed samples - comparing the size, shape, arrangement, and staining reactions of several different kinds of cells.

Instructions

You will work in pods (groups of four) and stain eight different samples. Each sample needs to be applied to a glass microscope slide and **heat fixed**. Then stain each sample using the steps described in the table above (rinse the sample briefly with water between each step). Blot dry at the conclusion of your staining procedure using bibulous paper and view with oil immersion.

Each person in your group must stain two of the following samples. You are responsible for recording accurate representations of all eight samples in your notebook. The samples that you will be observing consist of the following mixed cultures:

A) *Staphylococcus aureus* and *Klebsiella pneumoniae*

B) *Staphylococcus* aureus and Candida *albicans*

C) *Enterococcus faecalis* and *Moraxella catarrhalis*

D) *Enterococcus faecalis* and *Klebsiella pneumoniae*

E) *Bacillus cereus* and *Moraxella catarrhalis*

F) *Bacillus cereus* and *Rhodospirillum rubrum*

G) *Candida albicans* and *Rhodospirillum rubrum*

H) Desquamated cheek cells

The cheek cells can be obtained by scraping the inside of your cheek with a clean toothpick (or your fingernail). Apply the cells to a clean microscope slide, heat fix, and stain as described above.

Notebook Entry

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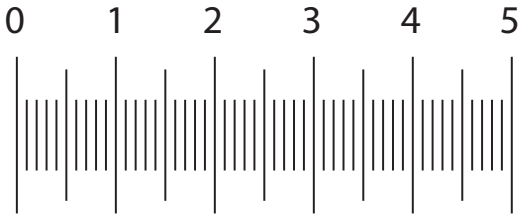
✪ Observations

• Carefully record the size, shape, arrangement, and color of the cells in the eight samples. This includes the two that you personally stained as well as the other six samples done in your pod.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the eight different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.

Specimen:
Total Magnification:



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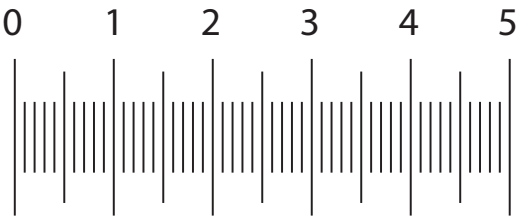

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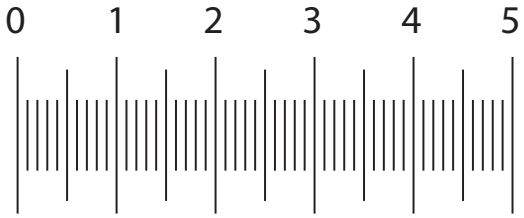

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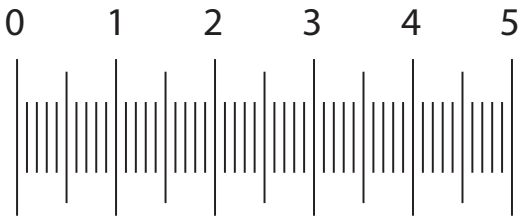

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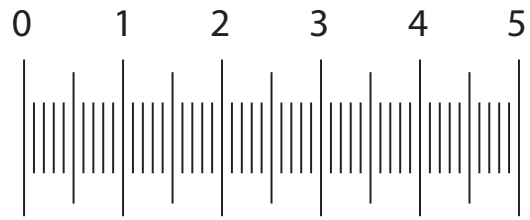

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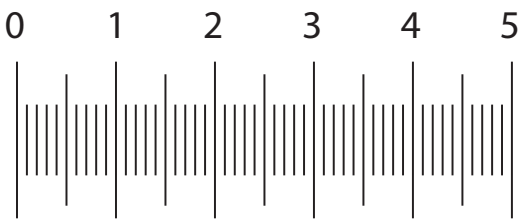

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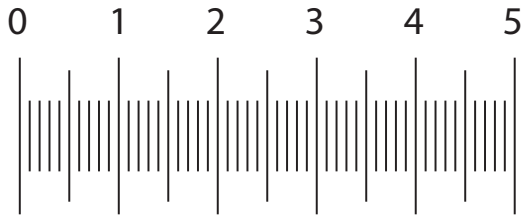

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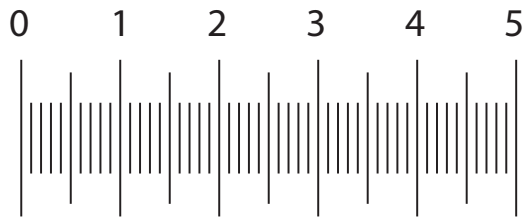


✪ Conclusions

1. List all of the prokaryotic cells seen in this exercise:

2. List all of the eukaryotic cells seen in this exercise:

3. Number the following cells from largest (1) to smallest (5) in size:

___ *Bacillus cereus*
___ *Moraxella catarrhalis*
___ *Candida albicans*
___ Cheek cells
___ *Klebsiella pneumoniae*

4. Give the 1) size in μm, 2) shape, 3) arrangement, and 4) Gram stain reaction for each of these microbes using proper terminology.

Bacillus cereus _____

Moraxella catarrhalis _____

Candida albicans _____

Enterococcus faecalis _____

Klebsiella pneumoniae _____

Rhodospirillum rubrum _____

Staphylococcus aureus _____

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your microscope slides in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Bacillus cereus* (BSL-1). This is a common soil microbe. It forms endospores.
2. *Moraxella catarrhalis* (BSL-1) This is commonly found on your skin and in your nose.
3. *Candida albicans* (BSL-1) This yeast is a member of our body's normal microbiota.
4. *Enterococcus faecalis* (BSL-2) This microbe is commonly found in animal feces.
5. *Klebsiella pneumoniae* (BSL-2) Strains of this microbe can cause pneumoniae in kids.
6. *Rhodospirillum rubrum* (BSL-1) This is a phototrophic microbe from the soil.
7. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed your stains.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

06

Capsule Stain

Background

Most microorganisms are covered by a layer of polysaccharide. This layer varies greatly in its extent between species. Very thin – difficult to detect – layers are referred to as a glycocalyx. Much more robust layers are called capsules. The primary function of a microbial capsule is to evade phagocytosis. Secondary functions of capsules include aggregation of cells, biofilm formation, and attachment to some surfaces. Most mucosal pathogens employ capsules as one of their main virulence factors.

The capsule stain does not actually stain the capsule. Instead, India ink is spread on the slide. The ink is too large to enter the cell and is excluded by the capsule too. We will visualize the cell bodies in this exercise using a simple stain (crystal violet). When correctly performed, your slides should have a dark black background (ink), purple rods or cocci (microbial cells), and clear areas around the cells (the capsule).

Instructions

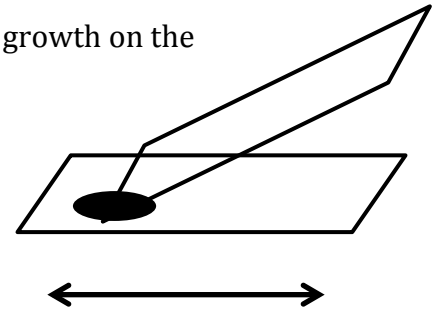
One person in your pair will work to visualize the capsules formed by both *Klebsiella pneumoniae* and *Cryptococcus neoformans*. Complete these steps for each isolate.

Put a loop of water at one end of a clean microscope slide. (drop of *K. pneumoniae*)

Flame sterilize a loop and touch the *Cryptococcus neoformans* growth on the slant.

Briefly mix the microbe in the drop of water on the slide.

Add one drop of India ink to the microbial suspension and make a smear using another slide as indicated here.



Air dry, but do not heat fix. Then cover the slide with crystal violet for one minute. Gently rinse the slide and allow to air dry. View your preparations using the oil immersion objective.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

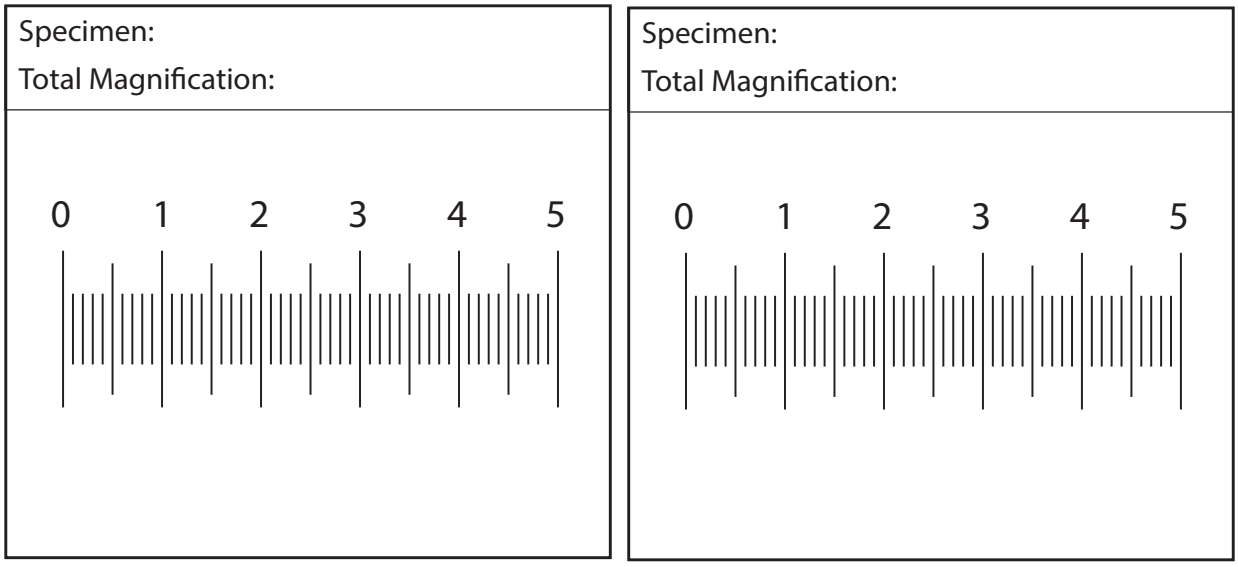
✪ Observations

• Carefully record the size, shape, arrangement, and color of the cells in your capsule stains. Indicate (using arrows) the cells, the India ink background, and the capsules. You should have one observation for each microbe.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the eight different samples. Make sure that you clearly label your samples.

2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.



✪ Conclusions

1. Which of these microbes is a eukaryote?
2. What group (kingdom or domain) of microbes includes *Klebsiella pneumoniae*?
3. What group (kingdom or domain) of microbes includes *Cryptococcus neoformans*?
4. What type of diseases do these microbes cause and why do they both make capsules?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your microscope slides in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Cryptococcus neoformans* (BSL-2). This is a common environmental microbe. It can be transmitted in bird feces.
2. *Klebsiella pneumoniae* (BSL-2) Strains of this microbe can cause pneumoniae in kids.

Return the microbial cultures when you have completed your stains.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.

5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

07

Endospore Stain

Background

Bacterial endospores are survival cells. Some soil bacteria that are members of the Firmicutes (primarily in the genus *Bacillus* and *Clostridium*) readily form them under adverse conditions. Endospores are produced in a form of cellular differentiation in response to starvation conditions. Once formed, endospores can withstand chemical agents, antibiotics, heating, cold, and even some exposure to radiation. Unfortunately, they are also resistant to staining. Today we will perform our second differential stain to visualize these structures. The procedure for the stain is shown below and is a modification of the original method devised by Dorner in 1922. In our stain today, we are going to use the heat of a steam bath to drive the primary stain (malachite green) into the tough endospore walls. Once stained, the spores are very difficult to destain. Therefore - unlike the Gram stain - , you may apply the water colorization step for as long as you like.

Step	Compound	Time	Endospore	Vegetative cell
Primary Stain	Malachite Green		Colorless	Green
Mordant	Heat	15 minutes	Green	Green
Decolorizer	Water	1 minute	Green	Colorless
Secondary Stain	Safranin	1 minute	**Green**	**Pink**

Instructions

One person in your pair will stain a sample of *Bacillus cereus* today from two kinds of media (Brain Heart Infusion Agar and Tryptic Soy Agar). One of these media is *much* richer in nutrients than the other. Your can stain both preparations on one microscope slide. For each culture, you should perform the following steps:

- Put a loop of water on one slide of a clean microscope slide.
- Flame sterilize a loop and add a small quantity of the *Bacillus cereus* culture.
- Allow the sample to air dry and heat fix.
- Perform the steps of the endospore stain from the previous table.
- Blot dry with bibulous paper.
- View your specimens using the oil immersion objective.

Notebook Entry

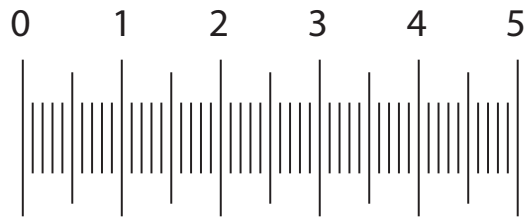
To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

- Carefully record the size, shape, arrangement, and color of the cells in your samples. Search around your slide and make sure that you see both the vegetative cells and the endospores.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the eight different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.

Specimen: Total Magnification:	Specimen: Total Magnification:
	

✪ Conclusions

1. What color would these vegetative cells be in the Gram stain?
2. List three diseases that are associated with endospore-forming (your may need to refer to the internet or your textbook.
3. Since boiling is insufficient to kill all endospores, how do we eliminate them from our laboratory media and equipment?
4. Was there a difference in the endospore formation on these media? If so, what might account for this difference.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your microscope slides in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Bacillus cereus* (BSL-1). This is a common soil microbe. It forms endospores.

Return the bacterial culture when you have completed your stains.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

08

Acid-Fast Stain

Background

The Kinyoun stain is a type of differential stain used to visualize acid-fast bacteria. This will be the third, and final, differential stain that will be used in our labs. The waxy mycolic acids in the cell wall of these microbes prevents most stains from directly binding to them. This procedure is often used to detect pathogens like *Mycobacterium tuberculosis* and *Mycobacterium leprae* in clinical samples. There are other types of acid fast stains as well such as Auramine-rhodamine (a fluorescence stain) and the Ziehl-Neelsen stain (which uses heat as a mordant). The primary stain and mordant are applied together in this protocol. At least 15 minutes is required for decent staining. Longer is better.

Step	Compound	Time	Acid-fast	Non Acid-fast
Primary Stain	Basic fuchsin	15 minutes	Colorless	Red
Mordant	Phenol		Red	Red
Decolorizer	Acid alcohol	~10 seconds	Red	Colorless
Secondary Stain	Methylene blue	1 minute	**Red**	**Blue**

Instructions

You will work individually to perform this stain on a mixture of an acid-fast cell (*Mycobacterium smegmatis*) and a non acid-fast cell (*Staphylococcus aureus*).

- Put a loop of water on a clean microscope slide.
- Flame sterilize a loop and add a small quantity of *Mycobacterium smegmatis* (acid-fast bacilli) to the water. Spread the cells out well; the waxy mycolic acids are repelled by water, so this takes some effort. Spend *at least* a minute trying to break up any chunks.
- Flame sterilize a loop and add a really small quantity of *Staphylococcus aureus* (non-acid-fast cocci) to the same slide. Spread these out too.
- Air dry and heat fix the mixed sample.
- Perform the acid-fast stain using the procedure in the table above.
- Rinse your slides with water between each step and blot dry with bibulous paper when complete.
- View the cells using your oil immersion objective.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

• Carefully record the size, shape, arrangement, and color of the cells in your samples. Search around your slide and make sure that you see both the acid-fast and the non acid-fast cells.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the eight different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.

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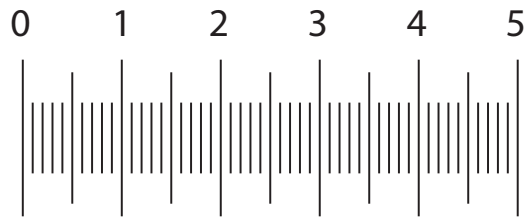
	

✪ Conclusions

1. Acid-fast cells often clump up on the slides. Why might that be?
2. List three diseases that are associated with acid-fast bacteria (your may need to refer to the internet or your textbook.
3. How is the appearance of an acid-fast bacillus similar or different from the appearance of a gram negative bacillus?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your microscope slides in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Mycobacterium smegmatis* (BSL-1). This is a fast growing acid-fast microbe. It can be found on healthy human skin and is rarely pathogenic.
2. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed your stains.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

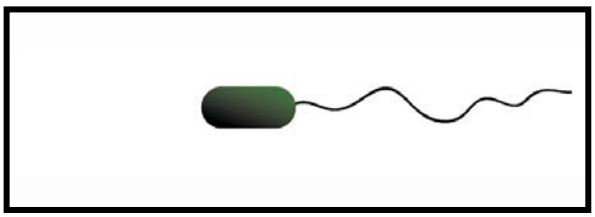
You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

09

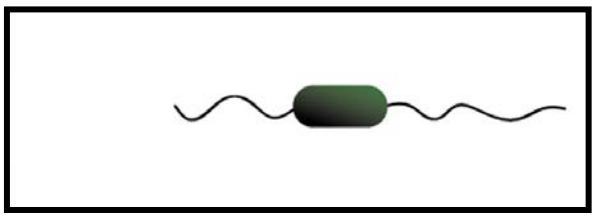
Bacterial Motility

Background

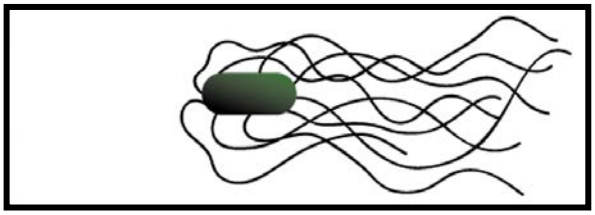
Flagella are external bacterial structures that enable motility in liquid media. They move the cells by rapidly rotating and providing thrust. Unfortunately, these structures are very thin and fragile. This make direct examination in the lab difficult. We will, therefore, only use prepared slides and observe motility directly. Flagella can be arranged in four different ways as shown below. You will see some of these arrangements today.



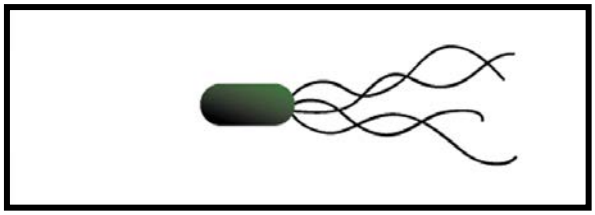
Monotrichous



Amphitrichous



Peritrichous



Lophotrichous

Instructions

Observe each of the commercially prepared flagella slides using your oil immersion objective.

In addition, you should observe the blood agar plates inoculated with *Proteus mirabilis* and *Klebsiella pneumoniae*.

Finally, you will need to prepare hanging drop preparations of *Klebsiella pneumoniae* and *Proteus mirabilis* cells to observe their motility.

- Use a toothpick to put a dab of petroleum jelly on the corners of a two coverslips.
- Put a loop of one culture in the center of a coverslip.
- Place a concave slide over the coverslips so that the culture is centered in the depression of the slide.
- Flip the slide over so that the coverslips are on top.
- View using your oil immersion (you will want to reduce the brightness of the light using the iris in the substage condenser).
- Try to determine which of the cultures (if any) are motile.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

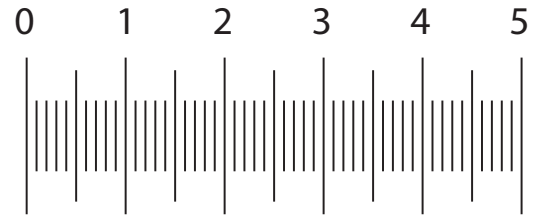
✪ Observations

• Carefully record the size, shape, arrangement, and color of the cells in your samples. Search around your slides and make sure that you see both amphitrichous and peritrichous flagella.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the eight different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.

Specimen:
Total Magnification:



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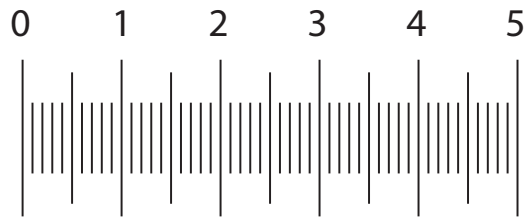


- Carefully record the appearance of the cultures on the blood agar plates. Once again, either produce a detailed drawing by hand or include photographs in your lab notebook. Please make sure that you clearly label your illustrations.
- Finally, you should describe what you saw in your two hanging drop slides. An illustration is not necessary since this is a kinetic observation (a movie would be more helpful – but difficult to get in a lab notebook.

✪ Conclusions

1. Why did the *Proteus mirabilis* plate look the way that it did (curled) in lab?
2. What flagellar arrangement does *Proteus mirabilis* exhibit?
3. Draw and label all four types of flagellar arrangements possible.
4. If *Klebsiella* is non-motile, why were the cells moving in the wet mount in lab?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your coverslips in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Commercial Slides

Do not throw the commercial slides or the hanging drop slides away! Wipe any immersion oil off of the slides using a Kimwipe. Then return the slide to the correct slide box. Return the hanging drop slides to the indicated container for later cleaning.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Proteus mirabilis* (BSL-2). This microbe is commonly found in soil and water. This species – though not this strain – accounts for about 90% of all *Proteus* infections.
2. *Klebsiella pneumoniae* (BSL-2) Strains of this microbe can cause pneumoniae in kids.

Return the bacterial culture when you have completed your stains.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

10

Protozoa

Background

The protozoa are single-celled, non-photosynthetic, primarily free-living organisms. While they do not possess a cell wall, many are covered by a series of flexible protein strips called the **pellicle**. The pellicle maintains the cell's shape and provides strength while remaining flexible. Members of the protozoa include several important human pathogens. These cells can reproduce asexually by binary fission either longitudinally (lengthwise) or laterally (widthwise). Some members of the protozoa can also undergo a type of sexual reproduction. The protozoan life cycle is **polymorphic** (having more than one stage). The infective form, or resting form, is the **cyst**. This stage is resistant to heat, acid, and drying – but less so than a bacterial endospore. If the cyst is ingested, the active form - the **trophozoite** - will grow out. This stage actively grows and reproduces. Under unfavorable conditions, the trophozoites will form cysts, which rest in the environment until favorable conditions for growth are again encountered. In addition, many protozoa are also **pleomorphic** (having more than one trophozoite form). The protozoa have traditionally been classified into four groups based upon their mechanism of motility – the amoeba, the ciliates, the flagellates, and the non-motile organisms. However, recent taxonomic work has revealed the protists to be polyphyletic (they are split amongst many different groups that are only distantly related genetically). Some of the more important of these groups are discussed below.

The first group, the **Amoebozoa**, includes organisms that move using **pseudopodia** ("false feet") - namely the amoeba. The amoebae reproduce by binary fission and feed by

engulfing their prey with their pseudopodia. Pathogenic sarcodines include *Entamoeba histolytica*, which is the causative agent of amoebic dysentery, and *Naegleria fowleri*. **Cercozoa** are another group of amoeba that differs by having a calcium carbonate shell (called a test). The **plasmodial slime molds** are also related to the amoebas. Most of these organisms (Amoebozoa, Cercozoa, and slime molds) are free-living and non-pathogenic.

A second group of protozoa are the **Alveolata**. Members this clade possess a complex cortical structure containing alveoli, extrusomes, and a microtubule framework. These microbes can be further divided into three main sub-groupings. The first of these is the **Ciliophora**. These include organisms that move using **cilia** ("eyelashes"). Most of the ciliates are free living, including *Paramecium* and *Didinium*, which are common inhabitants of pond water. The Ciliophora reproduce asexually by longitudinal binary fission. The ciliates contain two nuclei. The macronucleus contains several active copies of the chromosomes, which are used by the cell for growth and metabolism. The smaller nucleus, the micronucleus, contains one quiescent copy of the chromosomes. This second copy is used during genetic exchange. In sexual reproduction, two ciliates fuse in a process called conjugation. During conjugation, the micronuclei are exchanged. The only parasitic ciliate is *Balantidium coli*. This ciliate, the largest protozoan found in the gut, causes diarrhea. **Dinoflagellates** and. **Apicomplexans** are the final sub-group of the Alveolata, and consists of many different **non-motile** protozoa. These protozoans are **obligate parasites** and cause several important human diseases. The **Apicomplexa** are characterized by the presence of several organelles at one tip of the cell and include *Plasmodium*, which is the causative agent of malaria, as well as *Toxoplasma* and *Sarcocystis*.

Discicristata or **Euglenozoa** are members of what was once referred to as **Mastigophora** - organisms that move using a **flagellum** ("whip"). These organisms reproduce either by lateral binary fission or a complex sexual cycle involving two different hosts. The cells have a deep oral groove and possess disk-shaped mitochondria. Several of these flagellates are

important human pathogens. The hemoflagellates affect the circulatory system of infected humans. This includes *Trypanosoma* and *Leishmania,* both of which cause severe human infections. The sexual cycles of these parasites occurs in an insect or arthropod vector. When a carrier bites a human, the blood stream is infected. The trophozoite of these organisms has several stages, and eventually new gametophytes are generated which are able to infect another insect when it takes a blood meal from an infected human. Other members of this group, like *Euglena gracilis*, are free-living.

Excavata includes organisms that do not have mitochondria. These include important pathogens such as *Giardia lamblia*. Normally, an individual ingests some of the infective cysts. When these reach the intestine, the trophozoite form emerges and reproduces, causing diarrhea. During growth, some of the cells re-encyst and are passed back into the environment with the feces. Trophozoites are unable to grow outside of the intestine, so those that pass out with the feces quickly die. A second example is *Trichomonas vaginalis*, which can cause vaginal infections. This organism has no cyst stage. Instead, the trophozoite form is directly transmitted by physical contact.

Microsporidia (Opisthokonta) comprise another group of non-motile protozoa. These potential pathogens apparently lack both microtubules and mitochondria. Like the Oomycetes, this group of organisms is now thought to be more related to the fungi than protists.

Instructions

Observe each of the five commercially prepared protozoa slides using your oil immersion objective.

In addition, you should observe the live (non-pathogenic) *Paramecium caudatum* and *Amoeba proteus* cultures with wet mounts.

- Use a toothpick to put a dab of petroleum jelly on the corners of a two coverslips.
- Put a drop of one culture in the center of a microscope slide.
- Place a the coverslips so that the culture is covered and centered.

- View using your either 100X or 400X (you will want to reduce the brightness of the light using the iris in the substage condenser).
- Try to determine which of the cultures (if any) is more motile.

Notebook Entry

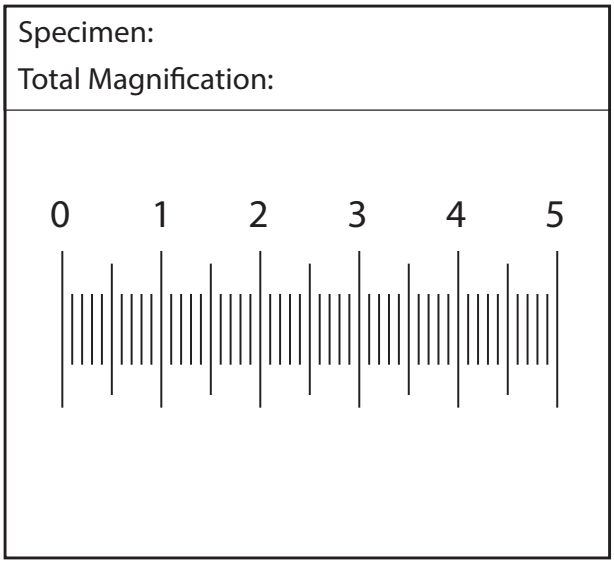
To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

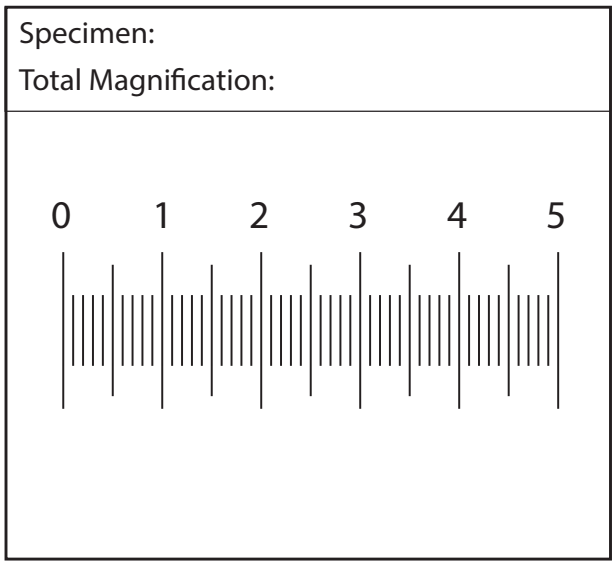
✪ Observations

• Carefully record the size, shape, arrangement, and color of the cells in your samples. Search around your slides and make sure that you see examples of all seven protozoa.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and microbial cells in focus for each of the seven different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.





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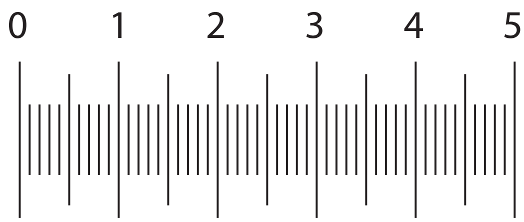

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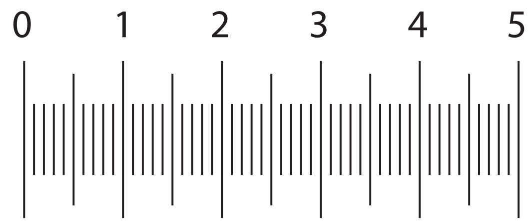

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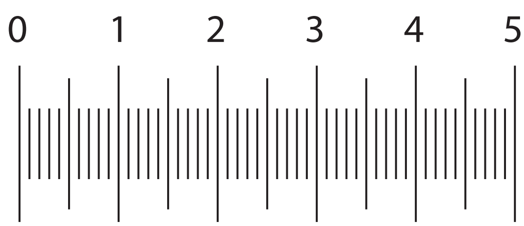

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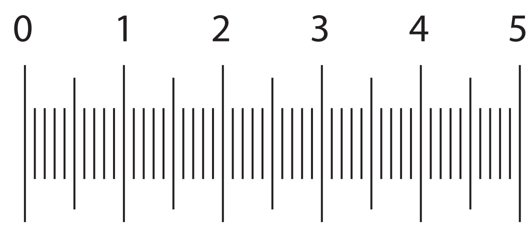

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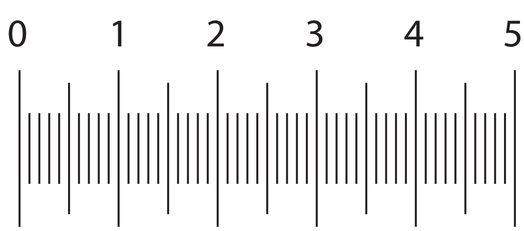

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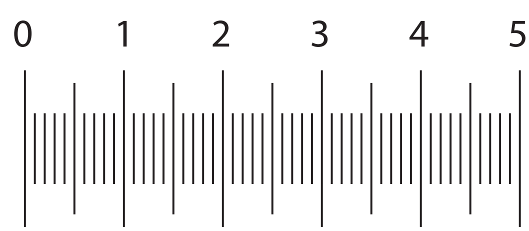


✪ Conclusions

1) Which of these is the largest in size? Which is the smallest?
2) What diseases (if any) are associated with each of these protists?
3) What phylogenetic groups do each of these protist belong to?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your non-pathogenic protozoa slides in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Commercial Slides

Do not throw the commercial slides or the hanging drop slides away! Wipe any immersion oil off of the slides using a Kimwipe. Then return the slide to the correct slide box. Return the hanging drop slides to the indicated container for later cleaning.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Paramecium caudatum* (BSL-1). This is a common ciliate from pond water.
2. *Amoeba proteus* (BSL-1) This is a common amoeba from pond water.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.

6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

11

Fungal Slide Culture

Background

The fungi are a group of eukaryotic microbes that can exist as single-celled or multicellular forms. Single celled fungi are referred to as **yeast**, while the multicellular forms are called **molds**, **mildews**, or even **mushrooms**. The cell wall of fungi is composed of **chitin**, a type of polysaccharide. The fungal membrane differs from ours in the sort of sterol that is incorporated; we use cholesterol in our membranes while the fungi use **ergosterol**.

The vast majority of all fungi are **saprophytes**. This is, they live by decomposing dead organisms. Due to their cell wall, fungi cannot use phagocytosis to take up their food. Instead, they secrete a variety of exoenzymes to break down larger molecules before transporting the smaller subunits into their cells. A few fungi can cause infections (called **mycoses**). These are usually limited to localized skin infections. Systemic infections by fungi are rare (and usually fatal).

The Kingdom Fungi consists of the Divisions **Chytridomycota**, **Zygomycota**, **Ascomycota**, and **Basidiomycota**. The latter three groups are primarily terrestrial and all were mentioned in lecture. The scientists that study these organisms are called **mycologists**.

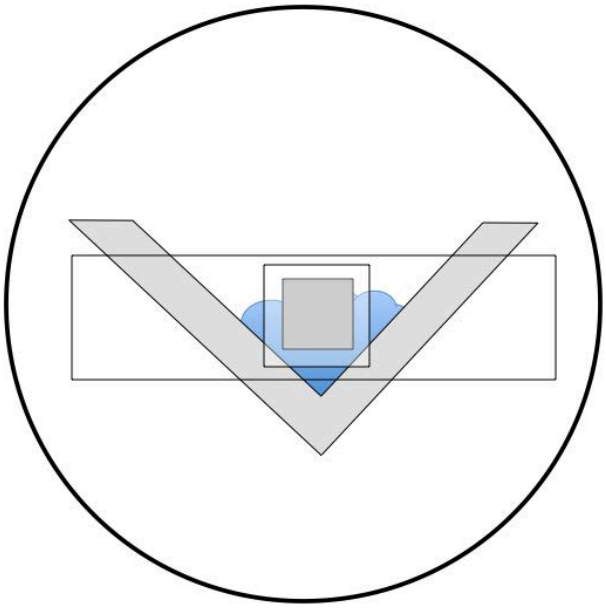
Multicellular fungi grow as filamentous structure termed a hyphae. In most fungi, hyphae are the main mode of vegetative growth, and are collectively called a mycelium. The hypha consists of one or more cells surrounded by a tubular cell wall. In most fungi, hyphae are divided into cells by internal cross-walls called **septa.** These hyphae are called **septate**. Septa are usually perforated by pores large enough organelles to flow between cells. Other fungi have aseptate hyphae, meaning their hyphae are not partitioned by septa. These are called **coenocytic**.

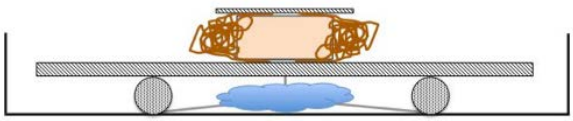
Viewing the a fungal mycelium with a microscope is difficult. The fungal mass is three-dimensional and our microscopes have a very limited focal plane. To make observations easier, we will perform fungal slide cultures. The point of the exercise is to coerce the fungi to grow as a thin layer to facilitate our observations later.

Instructions

You will work in groups of four for this exercise. Each person will receive a different coded fungal specimen. Each student will set up their own fungal slide culture as follows:

- Get an empty sterile petri dish and label the cover with your initials, section number, and fungal code number.
- Get a glass "V", disinfect it using an alcohol wipe and place it in the dish using a forceps.
- Get a clean microscope slide, disinfect it by briefly flaming, and place it on top of the glass "V".
- Obtain a piece of potato dextrose agar (approx. 1 cm x 1 cm) from your lab instructor and place it in the center of your microscope slide.
- Sterilize a teasing needle by flaming and allow it to cool. Inoculate the sides of the agar block. Re-sterilize the needle and return it.
- Finally, cover the agar block with a clean cover slip. You may disinfect it by briefly (very briefly) flaming.
- Add a small puddle of water (a few ml) to the dish in the space beneath your slide. Do not get water on your sample.
- Place your petri dish in the 20°C (room temperature) incubator and allow to grow for one week.





You will observe the fungi using your 40X objective after the cultures have grown.

- Get a clean slide and apply one drop of lactophenol cotton blue stain.
- Use a forceps to lift the coverslip off of your fungal culture.
- Place the coverslip right-side up onto the drop of stain.
- View your fungi with your light microscope.
- If the coverslip breaks, remove the agar block from your culture slide.
- Add a drop of lactophenol cotton blue stain where the block used to be.
- Cover the stain with a clean coverslip and view using your microscope.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

• Carefully record the size, shape, arrangement, and color of the cells in your samples. Search around your slides and make sure that you see both the hyphae and any asexual spores.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and fungal cells in focus for each of the eight different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.

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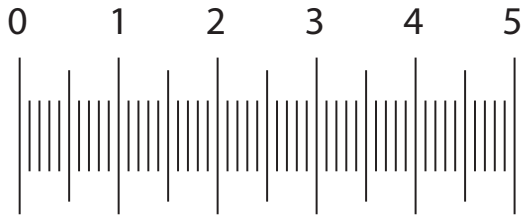

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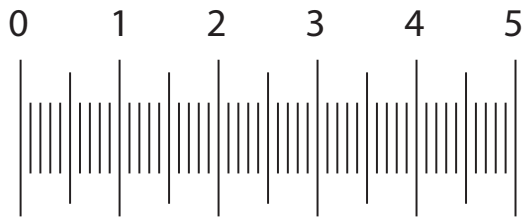

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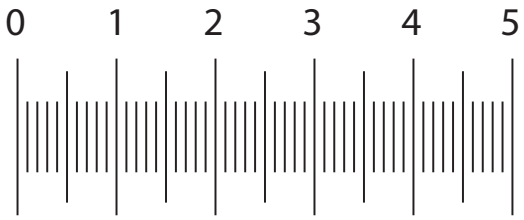

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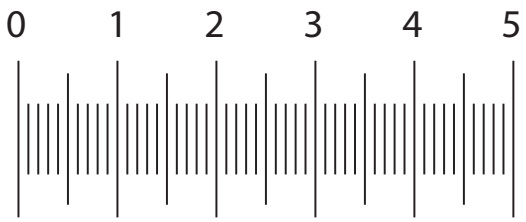


✪ Conclusions

1) Which fungus was associated with each of the numbers in your group?

2) Which of the four fungal divisions does each of your fungi belong to?

3) What type of asexual spores did you see for each fungus?

4) Why type of hyphae did each fungus have (septate/coenocytic)?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your slides and coverslips in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash. **Do not throw the glass Vs away**.

Fungal cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Rhizopus stolonifer* (BSL-1). This is black bread mold.
2. *Syncephalastrum racemosum* (BSL-1) A common soil fungus.
3. *Penicillium chrysogenum* (BSL-1). This microbe makes penicillin and can be found in blue cheese.
4. *Fusarium verticillioides* (BSL-1) Another fairly common soil fungus.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

Dispose of your petri dishes in the cans lined with an orange bag.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial

culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

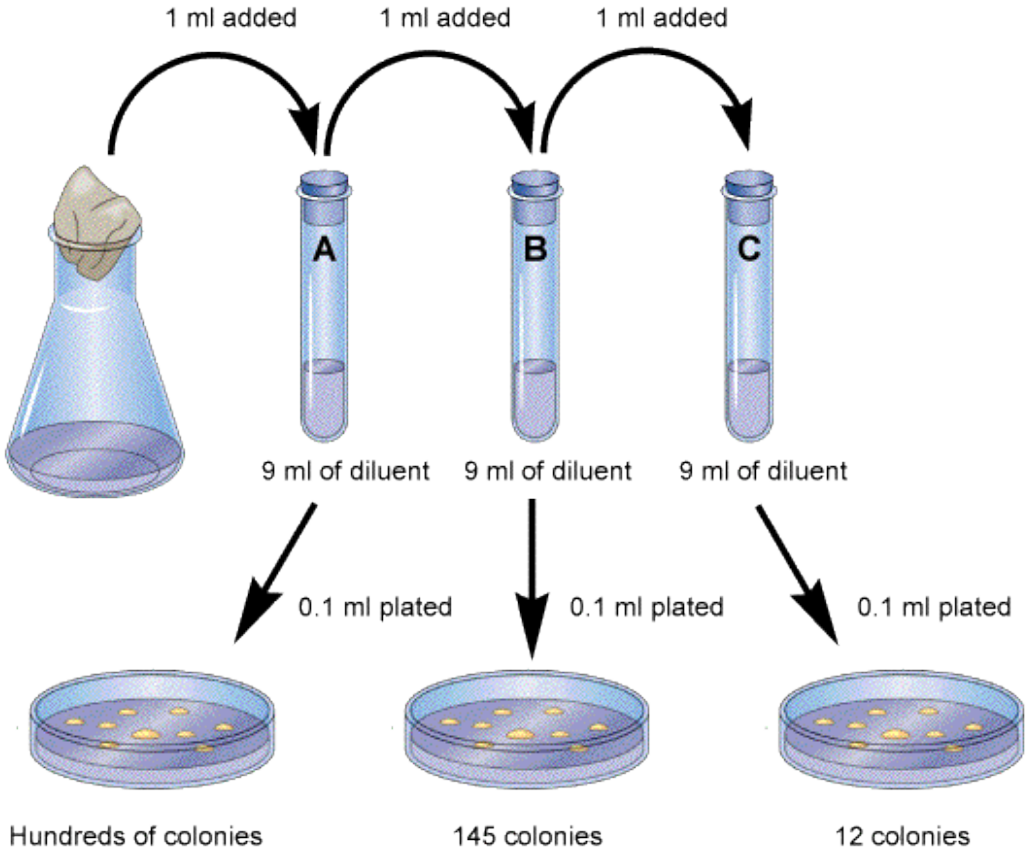
You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

12

Viable Bacteria Counts

Background

A common means of estimating the number of microbes in a liquid sample is to plate several known dilutions on agar plates and count the number of resulting colonies. A related method is used to enumerate viruses by counting plaques on a lawn of susceptible host cells. In either case, by finding one or more plates with a countable number of colonies, we may calculate the number of microbes present in the original stock. Typically, microbiologists seek plates with between 30 and 300 colonies or plaques. These numbers allow for statistical significance while still enable accurate counting of discrete and separate colonies. I refer to plates with 30-300 colonies "countable plates". For our stripes, a countable number would be between 10 and 30. An example of a dilution series is shown below.



In a dilution series, the original microbial suspension is sequentially reduced in several steps. Some people find the calculation of dilutions a little intimidating. Therefore, I will break this mathematic process down into a few simple parts. The first thing to consider is how much dilution is accomplished at each individual step of the serial dilution. The dilution factors of each step may be calculated for any volumes using the tube dilution factor (TDF) formula shown below.

$$Tube\ Dilution\ Factor\ (TDF) = \frac{Volume\ added + Diluent\ volume}{Volume\ added}$$

In the dilution series illustrated above, each of the tubes has the same dilution factor. Namely…

$$TDF = \frac{1\ ml + 9ml}{1\ ml} = \frac{10}{1} \equiv 10X \equiv 10^1$$

The series dilution factor (SDF) for any tube is merely the product of all tube dilution factors up to, and including that tube.

$$Series\ Dilution\ Factor\ (SDF) = TDF_A \bullet TDF_B \bullet TDF_C$$

Simply put, the SDF for tube C is 10 • 10 • 10 or 1,000. Note that when you <u>multiply</u> exponential numbers with the **same base**, you <u>add</u> the exponents ($10^1 \bullet 10^1 \bullet 10^1 = 10^3$).

The final dilution factor accounts for the fact that we often use more or less than one milliliter from our dilution tubes to inoculate the agar plates. If we choose to use an entire milliliter, this factor would be the same as the SDF. To account for the amount plated, we simply divide the SDF by the volume (in milliliters) used to inoculate the plate. As you can see in our example, each plate was inoculated with 0.1 ml so we would need to divide by that factor - x/0.1 (which is equivalent to 10X). Please note that the unit on the FDF is ml^{-1} (read this as per milliliter). The other dilution factors do not have units. The final dilution then, is simply the following.

$$Final\ Dilution\ Factor\ (FDF) = \frac{SDF}{Volume\ plated}$$

The FDF for plate "C" in our example is 1,000/0.1 ml = 10,000 ml^{-1} or 10^4 ml^{-1}. Likewise, the final dilution for plates A and B are 10^2 ml^{-1} and 10^3 ml^{-1}, respectively. We can now estimate the number of bacteria in our original culture using the final dilution factor for the plate giving a countable number of colonies (or plaques). The microbial estimate is the calculated as follows:

$$Microbes = \frac{CFU \bullet SDF}{Volume\ plated} = \frac{145 \bullet 100X}{0.1ml} = 145{,}000\ cfu/ml = 1.45x10^5\ cfu/ml$$

We can also do the opposite calculations – that is, given an approximation for the CFU/ml in a particular flask, we can predict the number of colonies (or plaques) that should be found on any plate. Using the illustration on page 1 again, let's assume that there are about 54,000 cfu/ml in the culture flask. How many colonies should be found on plate "C"? What about "B"? To find these answers, we <u>divide</u> the known CFU/ml by the SDF and <u>multiply</u> by the plated volume. Therefore…

$$B: \frac{54{,}000cfu/ml \bullet 0.1ml}{100} = 54\ colonies$$

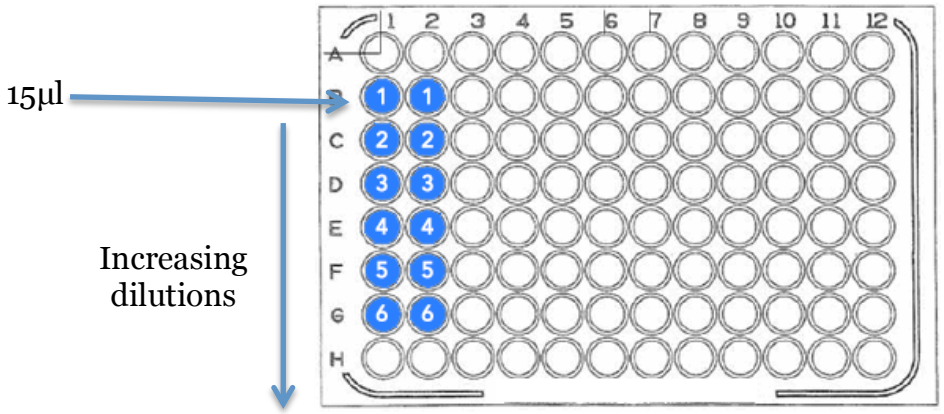
$$C: \frac{54{,}000cfu/ml \bullet 0.1ml}{1{,}000} \approx 5\ colonies$$

Instructions

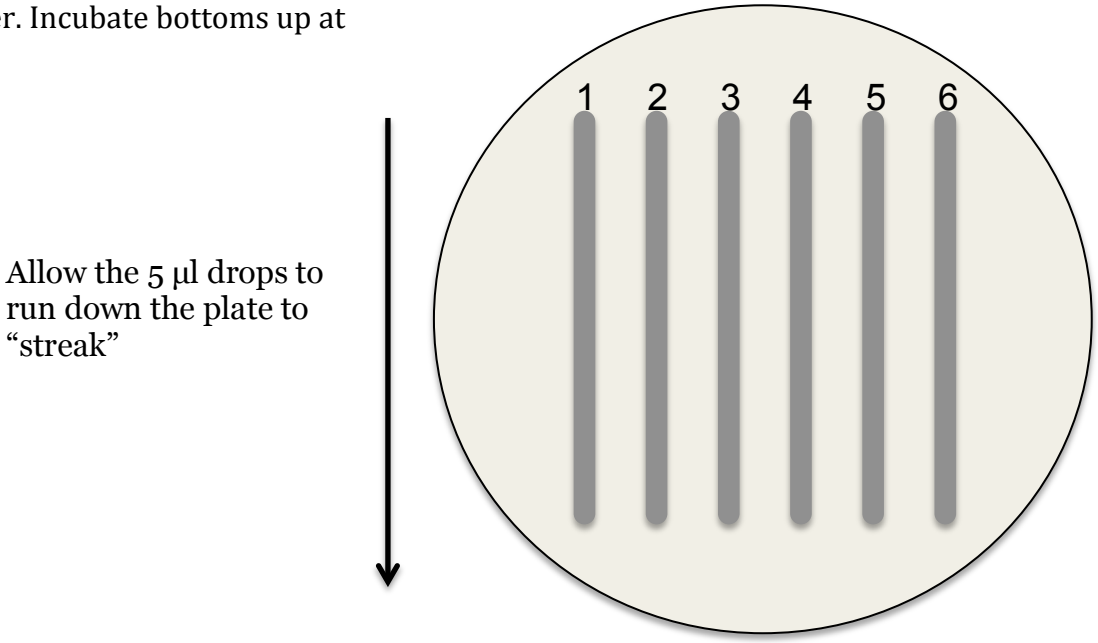
We will work in groups of four (pods) again today. Each group will perform duplicate dilutions of a bacterial culture to determine the number of cfu/ml. To do this, you will perform the following steps.

- Obtain a suspension of *Staphylococcus aureus* to enumerate.
- We are going to use microtiter plates to set up six 10^{-1} dilutions.
- Pour about 10 ml of sterile saline into a pipetting tray.
- Put six sterile micropipette tips on a multichannel pipetter (don't contaminate them with your fingers!)
- Transfer 135µl of saline (50µl + 50µl + 35µl) to 2 empty rows of wells as indicated below.
- Dispose of the used tips in a white plastic cup.

- Use a single channel pipetter to transfer 15µl of the bacterial suspension to the first dilution well.
- Mix by gently flushing in and out three times with the pipetter.
- Push all of the sample out of the pipette tip and remove from the well.
- Discard the contaminated tip into a white plastic cup.
- Use a fresh tip and repeat steps 7-10 but transfer from well 1 to well 2.
- Repeat to well 6.
- Use a second sample of the bacterial suspension to perform a second dilution series.



- Use a fresh set of six sterile pipette tips to transfer 5µl from each of the wells in one of your dilutions series to a plate of media. Let the liquid hang as small drops and touch them off onto the agar surface.
- Immediately tip the plate vertically so that the small drops run in parallel streaks.
- Repeat 14 and 15 with fresh tips and a new plate of media.
- Label the bottoms of your plates with your pod's symbol, section number, and dilution number. Incubate bottoms up at 35°C



Notebook Entry

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✪ Observations

• Carefully record the general appearance of your plates following incubation. Also record the number of colonies observed in any countable stripes.

You may record these data in one of two ways:

1) Draw a detailed picture of your plates' appearance. Make sure that you clearly label your samples and identify cfu numbers.
2) Take photographs of your plates and include them along with colony counts in your lab notebook.

✪ Conclusions

1. What was the tube dilution factor for well 1 in your dilution? Show your calculation.
2. What was the series dilution factor for well 6? Show your calculation.
3. What was the cfu/ml estimate from each of your dilutions? Show your calculations.
4. List three factors that might contribute to variations in the cfu/ml estimates between different groups in your lab section.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of any broken glass in the glass waste box when you are finished for the day.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

• *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed your stains.

Contaminated materials

Dispose of the contaminated micropipet tips in the orange bag-lined silver cans. Return the microtiter plates, sterile diluent, pipets, and unused tips when you are done with your dilutions. When you are finished observing the plates, they too should be disposed of in the orange biohazard bags.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

13

Bacteriophage Counts

Background

Bacteriophage are viruses of prokaryotic cells. As such, they are obligate intracellular parasites. Phage are very small (<200 nm) and therefore cannot be resolved with our light microscopes. We are going to enumerate (estimate the number of) a phage called T_4 in this lab exercise. T_4 is a **lytic** phage of *Escherichia coli* and is sometimes referred to as a coliphage. One virus particle that infects one bacterial cell will result in about 400 viruses being released when that infected cell lyses 100 minutes later. This is a much faster rate of replication than the bacterial rate (*E. coli* would make about 32 cells in the same time period). As a consequence, viral propagation can overwhelm the host cells. On solid media, this results in holes (called **plaques**) in the bacterial lawn.

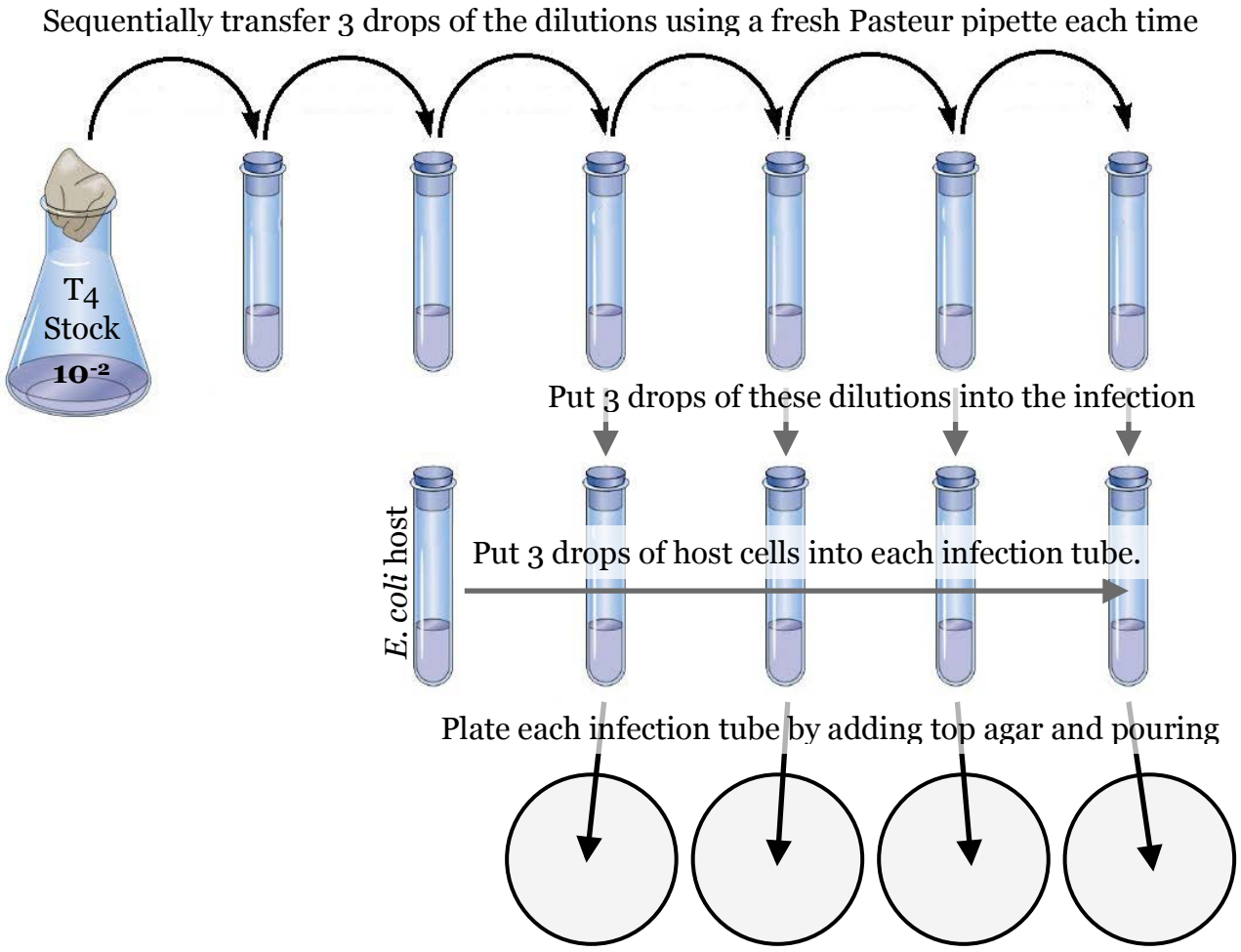
Viral plaques can be counted in much the same way that we counted *Staphylococcus aureus* colonies. These counts can be used to calculate the number of microbes present. We will serially dilute a viral stock in sterile diluent (peptone broth this time). The diluted viruses will be added to virus food (*E. coli* host cells) and plated. After incubation, plaques (areas of cell death) will appear on the plates. Even though we are counting areas of cell death instead of areas of cell growth, the math remains the same. You will report your findings in pfu/ml (plaque forming units per milliliter) instead of cfu/ml – for obvious reasons.

Instructions

We will work in groups today to dilute and plate a stock of bacteriophage T_4. Each pair will perform the following steps to determine the number of pfu/ml. Refer to the following figure for a graphic guide.

- Obtain a suspension of *Escherichia coli* host cells.
- Get six tubes of diluent (0.9 ml peptone broth) and label them 3 to 8.
- Have your lab instructor use a micropipette to transfer 100 µl of the 10^{-2} T_4 stock into dilution tube 3.

- Serially dilute bacteriophage T_4 by sequentially transferring 0.1 ml (3 drops) to each of the remaining diluent tubes.
- Get four empty infection tubes and label them 5 – 8.
- Add 0.1 ml of *Escherichia coli* host cells (0.1 ml) to each tube.
- Add 0.1 ml of diluted phage from the 10^5, 10^6, 10^7, and 10^8 dilutions to the corresponding infection tubes.
- Get four bottom agar plates and label each with the dilution (5, 6, 7, or 8), your section number, and your group's symbol.
- Add 7 ml molten top agar to an infection tube and immediately pour onto the surface of the corresponding bottom agar plate. Pour it on the agar surface! Tip it to spread the top agar evenly over the plate's surface.
- Set the plate aside for a few minutes to allow the top agar to solidify.
- Repeat this process for each of the infection tubes
- Incubate the solidified plates bottoms up at 35°C for 24 hours.



Notebook Entry

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✪ Observations

• Carefully record the general appearance of your plates following incubation. Also record the number of plaques observed on the plates.

You may record these data in one of two ways:

1) Draw a detailed picture of your plates' appearance. Make sure that you clearly label your samples and identify cfu numbers.
2) Take photographs of your plates and include them along with plaque counts in your lab notebook.

✪ Conclusions

1. What was the final dilution for your last plate? Show your calculation.
2. What was the series dilution factor for tube 3? Show your calculation.
3. What was the pfu/ml estimate from your dilution? Show your calculations.
4. List two factors that might contribute to underestimation of plaque counts from lower dilutions.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of any broken glass in the glass waste box when you are finished for the day. You should also dispose of any glass pipettes in the glass box.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

• *Escherichia coli* (BSL-1). This is a common member of your gut microbiota.

• Bacteriophage T_4 (BSL-1). This virus can only infect some strains of *E. coli*.

Return the bacterial culture when you have completed your stains.

Contaminated materials

Dispose of all of your used test tubes (dilution, infection, and pour tubes) in the unlined silver cans. Return any unused pipettes when you are done with your dilutions. Also, **do not** throw the pipette tubes away when empty; return them to the rack with blue tape. When you are finished observing the plates, they should be disposed of in the orange biohazard bags.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

14

Bacterial Growth Curve

Background

There are many ways to measure the growth of bacteria: direct cell counts, viable cell counts, protein determinations, etc. One of the most common techniques used in the lab, though, is spectrophotometry. Basically, this involves measuring the turbidity (cloudiness) of a bacterial suspension. Bacterial cultures obey Beer's law.

$$Absorbance = \epsilon \cdot L \cdot c$$

The absorbance (amount of light scattered) is directly related to three factors.

- The molar extinction coefficient (ε) is a constant that describes how much light is scattered per mole of cells. This value is constant is unknown for our cells and is affected by the size, shape, arrangement, and other optical qualities of the culture.
- The path length (L) is a constant that refers to the diameter of our spectrophotometer tubes.
- The concentration (c) is the only variable. As the number of cells increases, the absorbance will also increase.

Spectrophotometry has several advantages – it is fast, easy, and cheap. However, there are several limitations to this technique as well. The cells must be fairly concentrated to be measured (>10^6 cfu/ml). On the other hand, high density cultures(>10^9 cfu/ml) are difficult to measure too. Finally, both live and dead bacteria scatter light (so do non-bacterial particles) so turbidity can overestimate viable cell numbers.

The number of cells in a culture growing by binary fission can be calculated using the following formula (where X is the number of cells, Y is the number of doublings, and X_0 is the initial number of cells).

$$X = 2^Y \times X_0$$

We can then do a log transformation of that formula to arrive at this second equation.

$$\log(X) = \log(2) \times Y + \log(X_0)$$

The value of Y (the number of doublings) is equal to the time elapsed divided by the amount of time necessary for each doubling – the doubling time (g).

$$Y = {}^{t}/_{g}$$

Substituting in for Y, we now have…

$$\log(X) = {}^{\log(2)}/_{g} \times t + \log(X_0)$$

This is a linear function (y = mx + b). If we plot the logarithm of cell numbers versus time, the slope of the line will be…

$$m\ (slope) = {}^{\log(2)}/_{g} \ \therefore \ \ g = {}^{\log(2)}/_{m}$$

We can calculate the slope (rise over run) from the linear portion (the log phase) of our growth curve using two time points – P_1 and P_2 as follows:

$$\mathrm{m} = \frac{\log(OD\ of\ P_2) - \log(OD\ of\ P_1)}{time\ of\ P_2 - \ time\ of\ P_1}$$

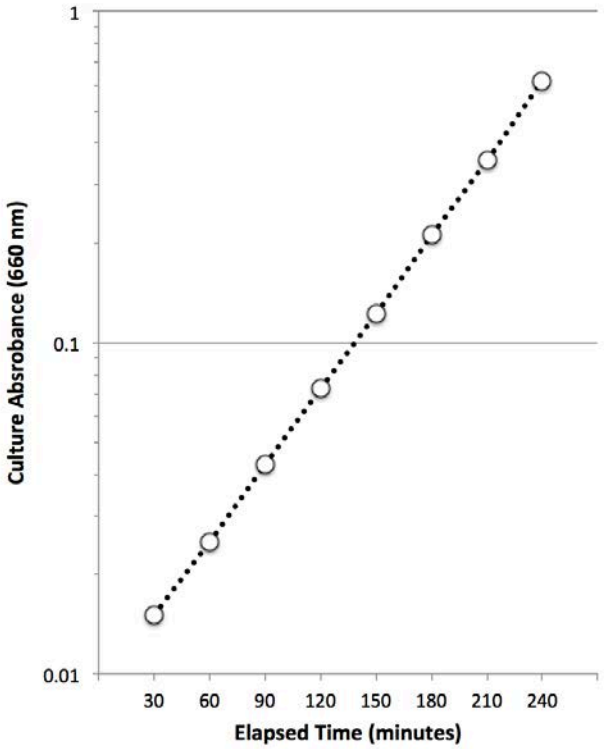
The growth rate (k) is the number of doublings that take place in a set time (like an hour). It is the reciprocal of the generation – or doubling time.

$$k = {}^{1}/_{g}$$

Time minutes	**Absorbance 660 nm**
30	0.015
60	0.025
90	0.043
120	0.073
150	0.122
180	0.211
210	0.355
240	0.615

Let's use this information to examine some practice data. The growth of a bacterial culture was followed for four hours by taking samples every thirty minutes and measuring the absorbance at 660 nm (deep red). The values recorded are given in the table below and are plotted on the next page. Notice how the y-axis appears (it is a log scale and not a linear scale). This semi-log plot is like plotting log(Absorbance) vs. time.

As you can see in this plot, all of the points fall on a line (the cells are in log phase). Therefore, we could use any two points to calculate g and k for this culture – let's use the first and last points (30 min, 0.015) and (240 min, 0.615). First we calculate m.



$$m = \frac{\log(0.615) - \log(0.015)}{240 - 30}$$

$$m = 0.00768\ min^{-1}$$

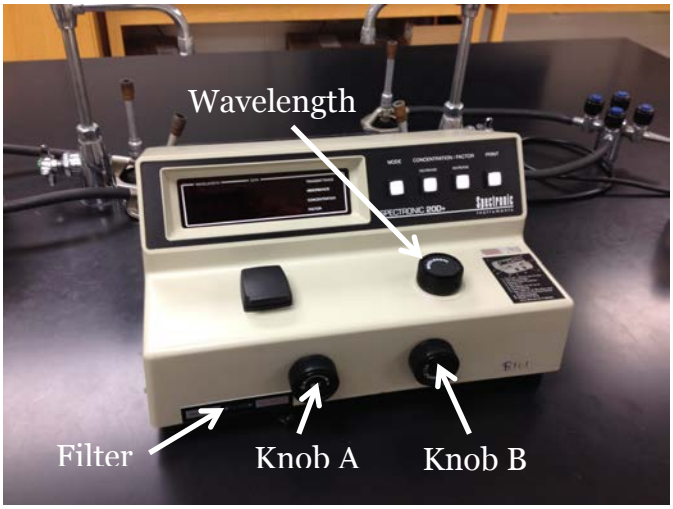
$$g = \frac{\log(2)}{m}$$

$$g = 39.2\ minutes$$

$$k = \frac{60}{g} \therefore k = 1.53\ hr^{-1}$$

Instructions

Six different groups will measure the growth of a simulated microbe, *Obnoxious oderium*, at one of six growth temperatures. You will need to gather the data from all six groups to complete your notebook entry. The first thing that you need to do is turn on the spectrophotometer at your station and zero it out. Refer to the picture below to perform the following steps.



- Set the filter range to 600 – 950 nm
- Turn the spectrophotometer on by turning **knob A**.
- Turn the wavelength knob to set the wavelength to 660 nm. Bacteria scatter red light most efficiently.
- With no samples in the meter and in transmission mode, use **knob A** to set the transmission to 0.00.
- Change the mode to absorbance.

- Resuspend your blank (0), wipe with a Kimwipe, insert into the sample chamber and close the sample lid. (I have the numbers on the tube face toward the front of the machine for consistency sake).
- Zero the absorbance of the blank using **knob B**.

You are now ready to read each of the growth samples at your station (30, 60, 90, 120, 150, 180, 210, and 240 minutes). **DO NOT OPEN THESE TUBES. DO NOT ADD ANYTHING OR REMOVE ANYTHING FROM THESE TUBES. DO NOT BREAK OR DISPOSE OF THESE TUBES!!**

- Resuspend your samples by inverting several times.
- Place a tube in the spectrophotometer and close the sample lid.
- After your reading stabilizes (several seconds) read the absorbance.
- Remove and save your samples.
- When all samples have been read, turn off your spectrophotometer using **knob A**.
- Go to the other five stations to collect your classmates data.

Notebook Entry

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✪ Observations

• Carefully record the absorbance data from all six stations in a table. An example table is given on the next page.

• Plot your six growth curves on semi-log paper or use a semi-log plot in Excel.

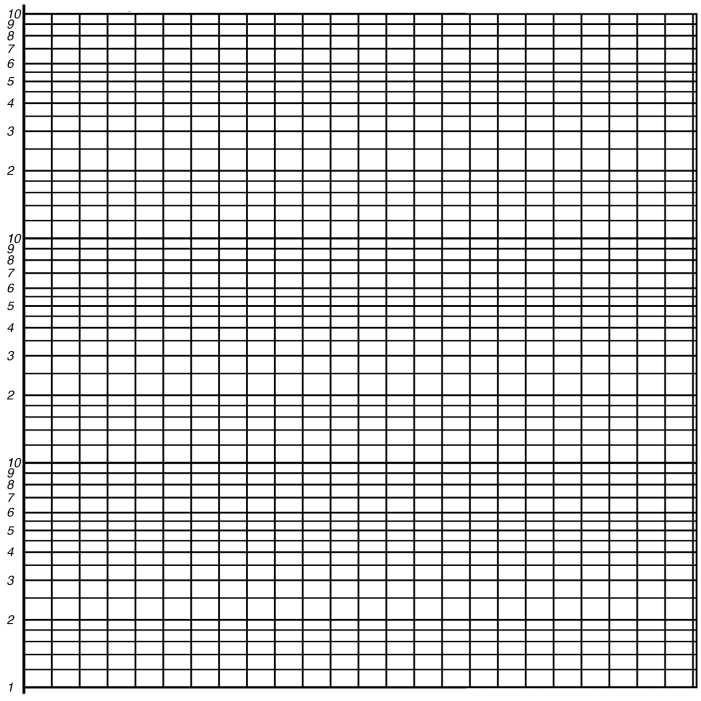
• Calculate the slopes of the linear portions of each plot.

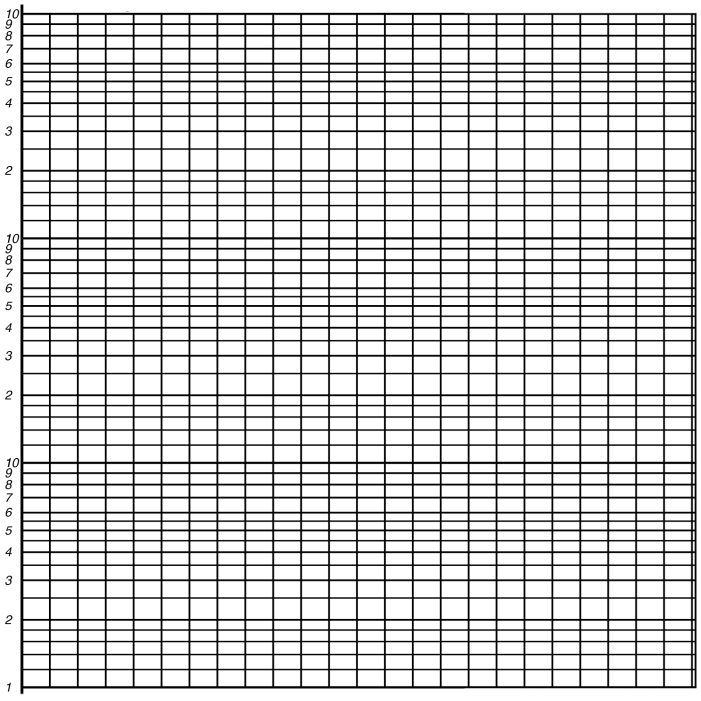
• Use the slopes to calculate g (in minutes) for each culture.

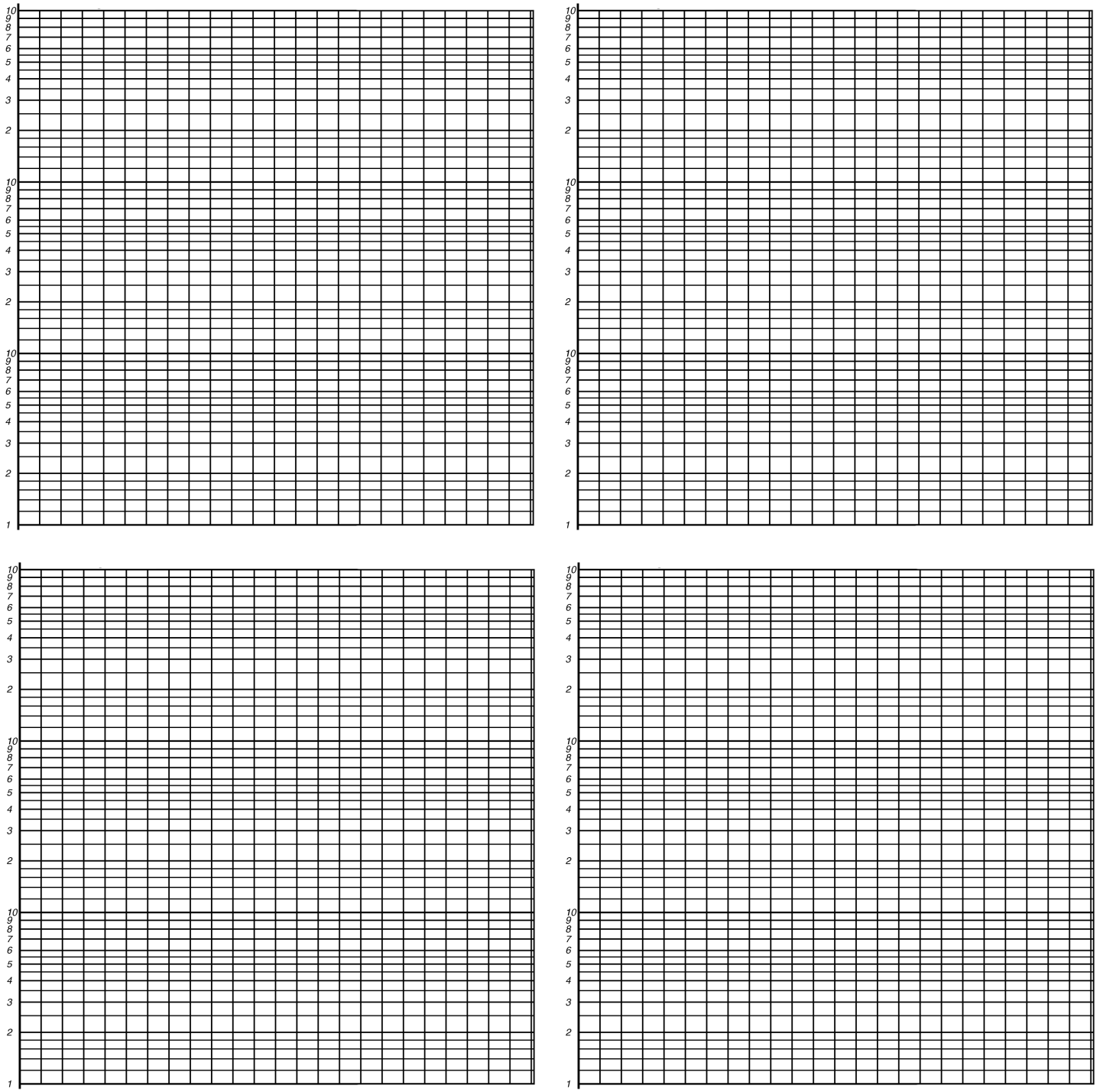
• Use the g values to calculate k (in $hours^{-1}$) for each culture.

• Plot k vs. temperature (k on y-axis and temperature on x-axis)

Time	20°C	25°C	30°C	35°C	40°C	45°C
30						
60						
90						
120						
150						
180						
210						
240						







✪ Conclusions

1. What phases of growth did you observe in your samples?
2. Which temperature had the fastest growth rate?
3. What type of growth (psychrophile, mesophile, thermophile) did this culture show?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Spectrophotometer samples

Do not throw the sample tubes away! Return your tubes to the test tube rack at your station. These samples contain barium chloride and a weak solution of sulfuric acid. If you manage to break or spill a tube, clean up with a copious amount of water. Also, let your lab instructor know.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

15

Environmental Conditions

Background

Microbes vary tremendously with regard to the environmental conditions that support their optimal growth. As a consequence, nearly every possible niche on Earth is currently populated with microbes. Each of these locations has their own chemical and physical conditions and supports microbes that favor that environment. In today's exercise, we will examine the affect of three physical parameters on microbial growth.

Temperature: Microbes that grow optimally under cool conditions (0-15°C) are called psychrophiles. Other microbes can tolerate cool conditions, but grow best around room temperature (20°C). These are referred to as psychrotrophs. Most microbial pathogens are mesophiles. These microbes grow best between room temperature and body temperature (20°-40°). Finally – some microbes like it hot. Microbes with optimal growth under high temperatures (>40°C) are called thermophiles.

pH: Microbial growth also varies with respect to the concentration of hydrogen ions in the environment. Remember that pH is the $-\log[H^+]$. Microbes that grow best at low pH (1-5) are called acidophiles. Neutrophiles grow best at neutral pH (6-8). Lastly, alkalinophiles are microbes that grow best at alkaline, or high, pH (9-14).

Salt: High osmolarity (lots of solutes) tends to be inhibitory for most microbes. The presence of high salt or sugar concentrations makes the environment hypertonic to the microbial cell. Water, therefore, tends to leave the microbial cell by osmosis resulting in cell death. Most bacteria are inhibited by moderate levels of salt (1-5%). These are termed non-halotolerant. A few microbes can tolerate higher concentrations (1-15%). These are called halotolerant. In contrast, some microbes actually require elevated salt concentrations for growth. These are the halophiles. The difference between a halophilic and halotolerant microbes is that the halotolerant can grow with no added salt. The halophile cannot.

Instructions

Your laboratory section will work in six groups today. Each group will be given one strain of microbe to test. You will evaluate the growth of your microbe at five different temperatures, five different pHs, and five different salt concentrations.

- Obtain the pure culture for your section.
- Get five temperature tubes (unlabeled broth tubes) and label them with your organism's initials and put a distinctive mark on each cap to make locating your tubes easier in the future. Label the tubes for the temperatures (5, 20, 35, 42, 55).
- Get five pH tubes (labeled P3, P5, P7, P9, and P11) and label them with your organism's initials and put a distinctive mark on each cap to make locating your tubes easier in the future.
- Get five salt tubes (labeled 0.5, 1, 3, 5, 10) and label them with your organism's initials and put a distinctive mark on each cap to make locating your tubes easier in the future.
- Use a Pasteur pipette to inoculate each of your 15 tubes. Add two drops of your pure culture to each tube.
- Place each of your temperature tubes into an incubator set to the corresponding value. Your lab instructor will tell you where they are.
- All of the pH tubes (**except for the *Bacillus stearothermophilus***) go into the 35°C incubator. The *Bacillus stearothermophilus* pH tubes should be placed in the 55°C incubator.
- All of the salt tubes (**except for the *Bacillus stearothermophilus***) go into the 35°C incubator. The *Bacillus stearothermophilus* salt tubes should also be placed in the 55°C incubator.
- After growth has occurred, resuspend your cultures by briefly vortexing.
- Obtain turbidity standards (0, 1, 2, 3, and 4). Shake them to resuspend the particles.
- Compare your cultures to the standards and estimate the turbidity (you can split the difference (i.e. 3.5) if you wish.
- Plot your observed values in the graphs provided.

Notebook Entry

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to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

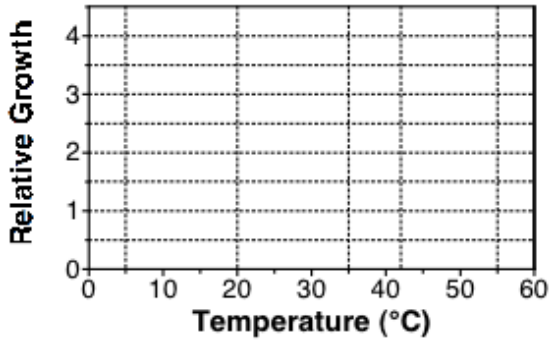
1. Record the turbidity for each of your 15 cultures.
2. Gather the data for the other 5 cultures in the lab.
3. Using the panels below, plot your 90 data points and connect with lines.

✪ Conclusions

1. Which microbe(s) was a psychrotroph?
2. Which microbe(s) was a meosphile?
3. Which microbe(s) was a thermophile?
4. Which microbe(s) was an acidophile?
5. Which microbe(s) was a neutrophile?
6. Which microbe(s) was a alkalinophile?
7. Which microbe(s) was halotolerant?
8. Which microbe(s) was non-halotolerant?

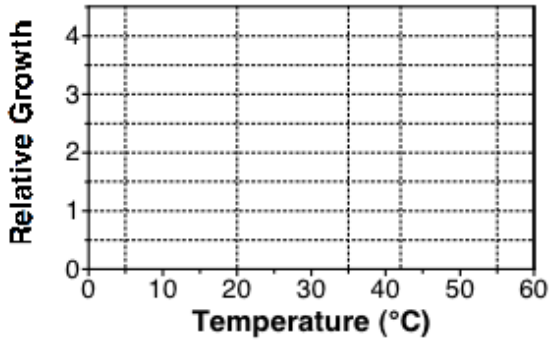
The Effect of Temperature on Microbial Growth

Alcaligenes faecalis



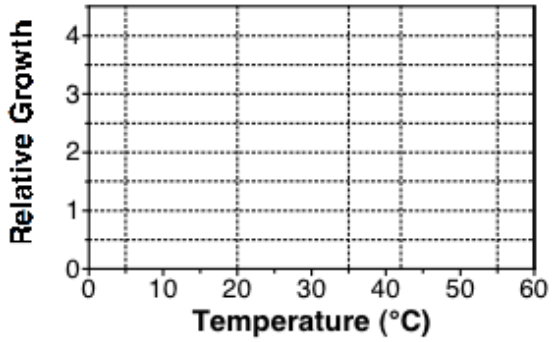
Type of growth:

Bacillus stearothermophilus



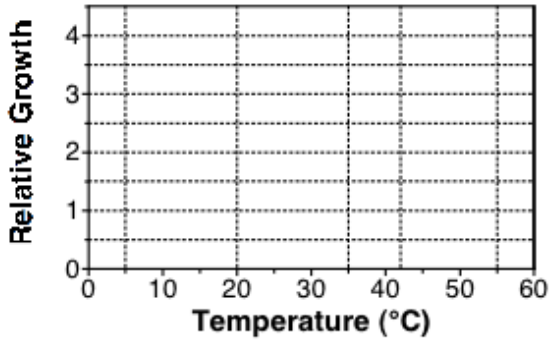
Type of growth:

Escherichia coli



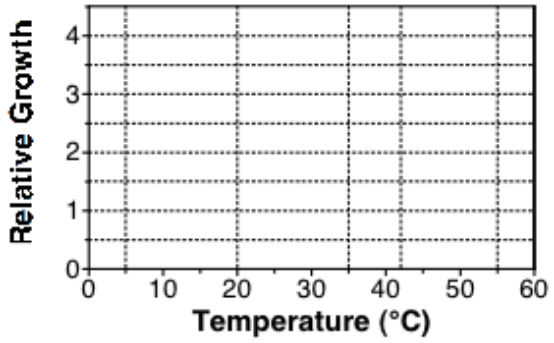
Type of growth:

Pseudomonas fluorescens



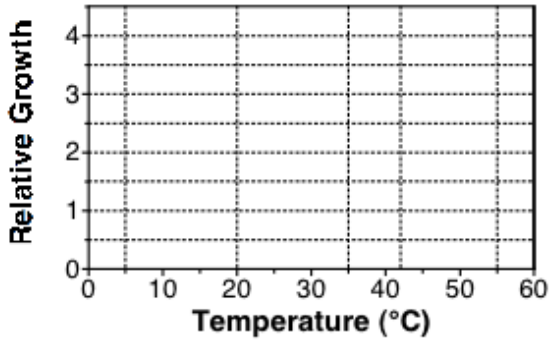
Type of growth:

Saccharomyces cerevisiae



Type of growth:

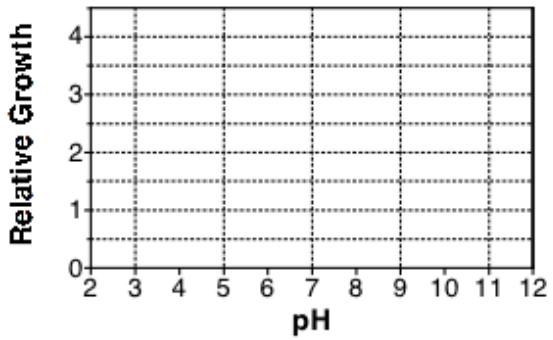
Staphylococcus aureus



Type of growth:

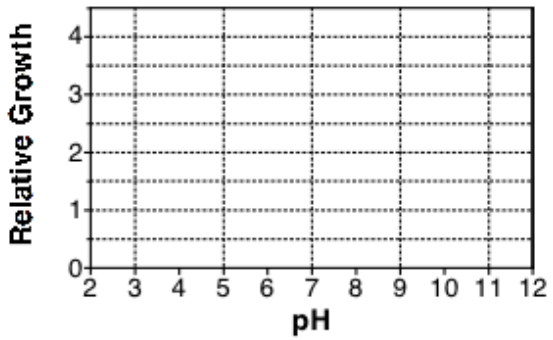
The Effect of pH on Microbial Growth

Alcaligenes faecalis



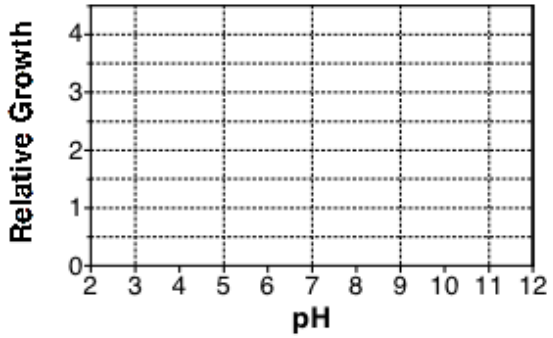
Type of growth:

Bacillus stearothermophilus



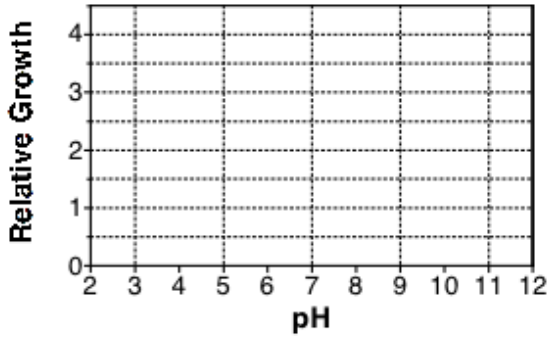
Type of growth:

Escherichia coli



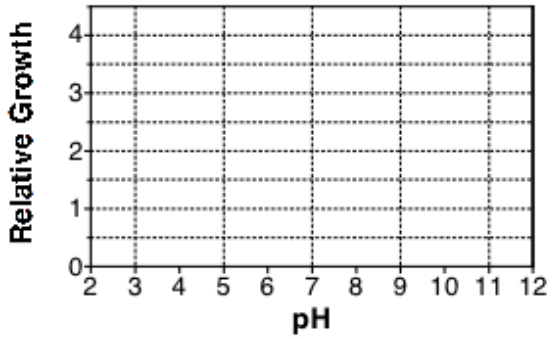
Type of growth:

Pseudomonas fluorescens



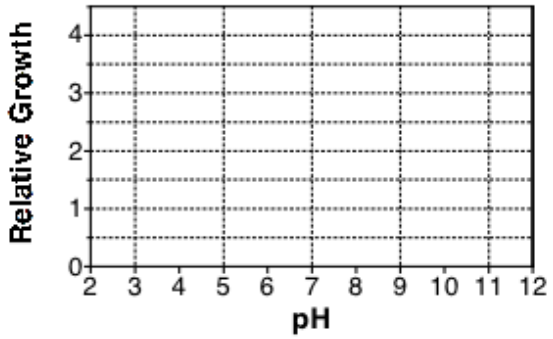
Type of growth:

Saccharomyces cerevisiae



Type of growth:

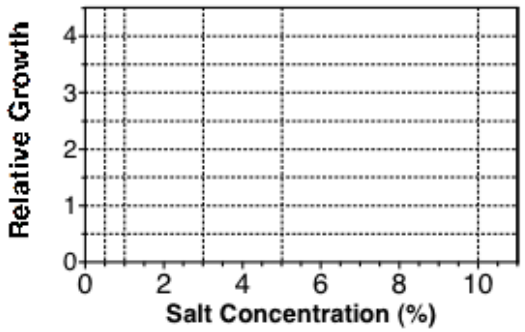
Staphylococcus aureus



Type of growth:

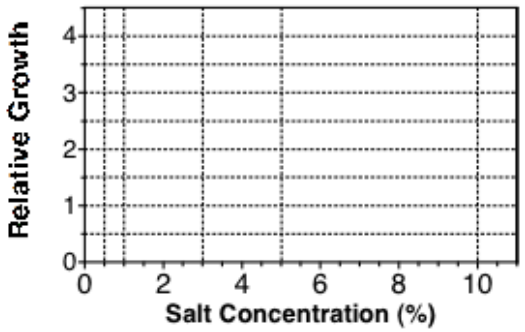
The Effect of Salt on Microbial Growth

Alcaligenes faecalis



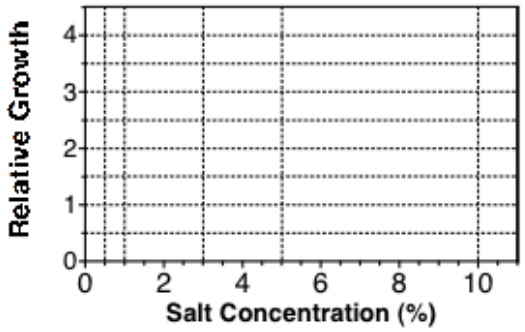
Type of growth:

Bacillus stearothermophilus



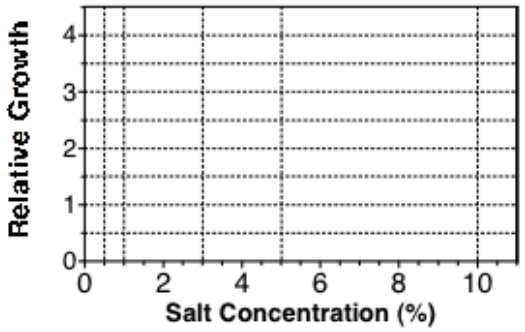
Type of growth:

Escherichia coli



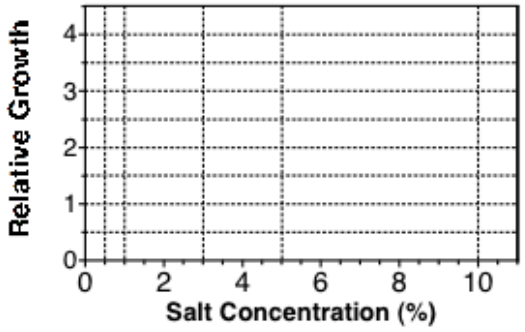
Type of growth:

Pseudomonas fluorescens



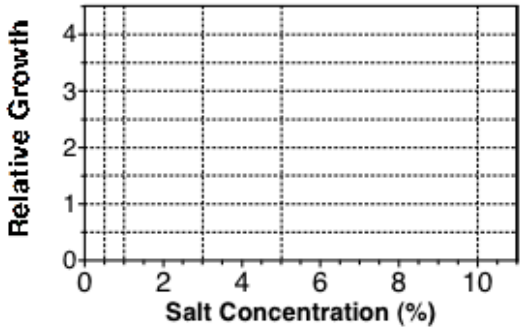
Type of growth:

Saccharomyces cerevisiae



Type of growth:

Staphylococcus aureus



Type of growth:

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your Pasteur pipette in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Alkaligenes faecalis* (BSL-1) A common environmental microbe that was first isolated from feces. It is abundant in other locations as well.
2. *Bacillus stearothermophilus* (BSL-1) A common endospore-forming soil bacterium.
3. *Escherichia coli* (BSL -1) The K12 strain is the most studies life form on Earth
4. *Pseudomonas fluorescens* (BSL-1) A common environmental isolate; rarely pathogenic
5. *Saccharomyces cerevisiae* (BSL-1) This is common baker's or brewer's yeast.
6. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed your inoculations.

Contaminated materials

All of your culture tubes should be placed in the unlined buckets at the front of the lab.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for

this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

16

Anaerobes

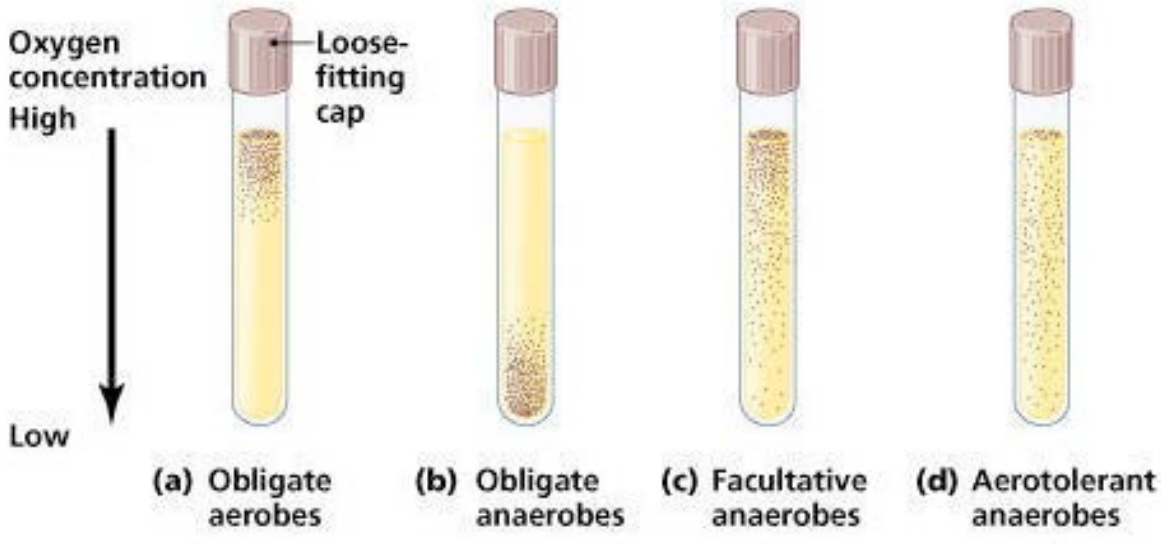
Background

Oxygen is a very chemically reactive element and is absolutely required for the growth of many organisms (like us!). However, during metabolism, oxygen can form several toxic intermediates that can kill or inhibit microorganisms. These include superoxide anion, hydrogen peroxide, and the hydroxyl radical. These three molecules can cause oxidative damage to lipids, proteins, and DNA in cells. The ability of microbes to detoxify these deleterious byproducts varies, and thus different bacteria prefer different oxygen tensions in their growth media.

Organisms that require oxygen to live are called obligate aerobes. Obligate anaerobes are unable to use molecular oxygen to obtain energy and are usually killed or inhibited by its presence. *Facultative anaerobes* can use oxygen if it is available but can also grow when oxygen is absent. In contrast, *aerotolerant anaerobes* cannot use oxygen for growth, but they do tolerate its presence well. Some bacteria require a lower oxygen concentration than what is in the air. These organisms are classified as *microaerophilic*. A variety of culture methods have been developed to satisfy these diverse oxygen needs. Thioglycolate broth, anaerobic jars, and candle jars create alternative environments to atmospheric oxygen.

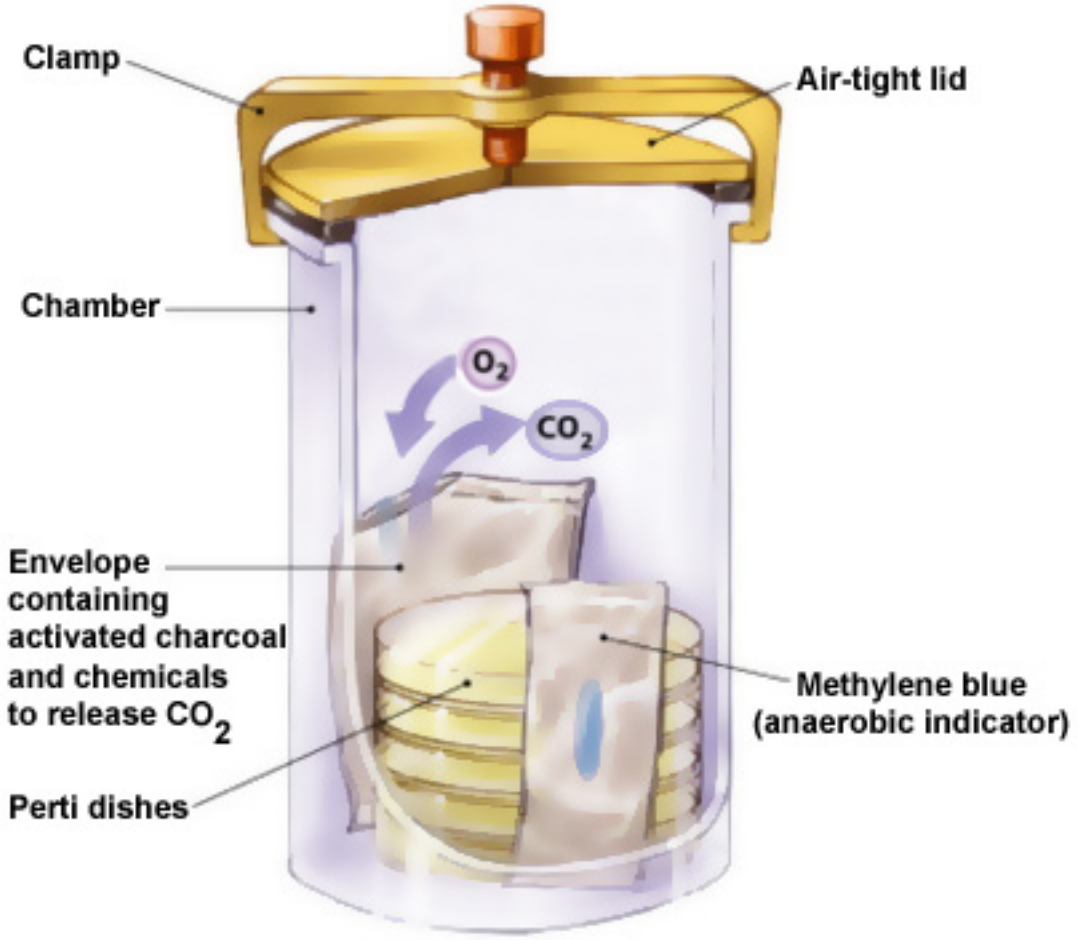
Thioglycolate Broth

Thioglycolate (thio) broth contains sodium thioglycolate and cysteine that act as reducing agents to create anaerobic conditions when they convert molecular oxygen to water. Dyes such as resazurin or methylene blue are usually added to the broth to provide a visual indication of the presence of oxygen. Resazurin is pink when oxidized and colorless when reduced. Methylene blue is blue when oxidized and colorless when reduced. Resazurin is present in the thioglycolate tubes that we will be using. The pink band near the top of the broth results when oxygen diffuses in. Strict aerobes will grow only in the pink band, microaerophiles will grow near the bottom of the band where the concentration of oxygen is lower. The absence of pink in the rest of the tube indicates the absence of oxygen and a



suitable environment for strict anaerobes. Both facultative anaerobes and aerotolerant anaerobes will grow throughout the tube; however, facultative anaerobes will grow most densely where oxygen is present.

Anaerobic Jar



The Brewers Anaerobic agar plates employed for culturing our organisms do not contain reducing agents. They can however be used to culture anaerobes if they are placed inside an anaerobe jar - a chamber from which the oxygen is removed. Commercially available gas generator packets (e.g. GasPak) are placed in the jar along with plates or tubes containing conventional media destined to be incubated anaerobically. Opening the packet causes a chemical reaction to take place:

Sodium citrate plus Sodium bicarbonate react to release carbon dioxide (CO_2)

Activated charcoal in the packet binds free oxygen gas (O_2).

As a result of these reactions, the oxygen within the jar is removed and replaced with a carbon dioxide atmosphere. In order to assure that oxygen actually was removed from the chamber, a strip of paper soaked in methylene blue dye is included in the jar. It is blue when exposed to oxygen but will become colorless (white) when oxygen is absent. The Brewers anaerobic agar also includes methylene blue. When you first streak the plates, they should be a pale blue color. After incubation in the jar, they should appear colorless.

Instructions

Your lab section will work in six groups again.

Each group will obtain **four** fluid thioglycollate medium (FTM) tubes. Please handle the FTM tubes carefully – keep the tubes in an upright position; do not invert or agitate the tubes during or after inoculation. Secure the caps tightly before incubation. **We do not want to introduce any more oxygen than is absolutely necessary.**

The Brewer Anaerobic agar (BAA) plates are at the side demonstration table – **each group needs two**.

The broth cultures of *Escherichia coli, Clostridium sporogenes, Enterococcus faecalis,* and *Pseudomonas aeruginosa* (one of each/group) will be provided.

The anaerobe jars are clearly labeled with the section numbers that will use them – when ready place each of the anaerobe jars into the 35°C incubator labeled for it in the lab. Do not charge a jar until the last section indicated on the jar has placed its BAA plates into it.

Label the Brewer Anaerobic agar plates and divide the bottom into quarters. Streak each organism onto one quarter of the plates. Place one Brewer Anaerobic agar plate into the GasPak jar (bottoms up) and the other into the aerobic 35°C incubator (bottoms up).

Flame a loop, let it cool, and use it to obtain a small quantity of each broth culture. Insert the loop all the way to the bottom of a thioglycollate tube and gently swirl it around a little bit. Then, slowly withdraw the loop while continuing to gently swirl it. Mix the tube by rolling between your hands for about five seconds. Place your labeled and inoculated tubes into the provided rack in the 35°C incubator.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

• Carefully record the growth in your thioglycollate tubes and on your BAA plates.

You may record these data in one of two ways:

1) Take pictures using your camera. Tape the pictures into your lab book and label them well.
2) Carefully draw the appearance of your cultures in your lab book. Carefully label each illustration.

✪ Conclusions

1. *Clostridium sporogenes* was a(n) _____
2. *Pseudomonas aeruginosa* was a(n) _____
3. *Enterococcus faecalis* was a(n) _____
4. *Escherichia coli* was a(n) _____

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Escherichia coli* (BSL-1). The K12 strain is the most studied organism on the planet.
2. *Clostridium sporogenes* (BSL-1) This is basically *Clostridium botulinum* without toxins.
3. *Enterococcus faecalis* (BSL-2) This is an indicator strain for animal feces in ground water.
4. *Pseudomonas aeruginosa* (BSL-2) This is commonly found in wet environments and can be a potent pathogen under the right circumstances.

Return the bacterial culture when you have completed your stains.

Contaminated materials

Dispose of your BAA plates in the silver cans lined with the orange bags. Your fluid thioglycollate tubes should be put in the unlined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

17

Antimicrobial Compounds

Background

We often wish to kill or inhibit the growth of microbes in our environment. Today, we will be examining three types of antimicrobial compounds and compare their effectiveness using the disk diffusion assay.

The first group of chemicals that we will employ are **disinfectants**. These compounds are used on inanimate objects (telephones, desks, etc.) and tend to be more toxic. The chemical that we are using represent some of the chemical classes mentioned in lecture.

Label	Chemical	Class
L	Lysol	Phenolic
R	Roccal	Quaternary ammonium compound
B	Bleach	Oxidizer
A	Aldehyde	Alkylator

The next group of chemicals that we will employ are **antiseptics**. These compounds are used on living surfaces (like your skin) and tend to be much less toxic. The chemical that we are using represent some of the chemical classes mentioned in lecture.

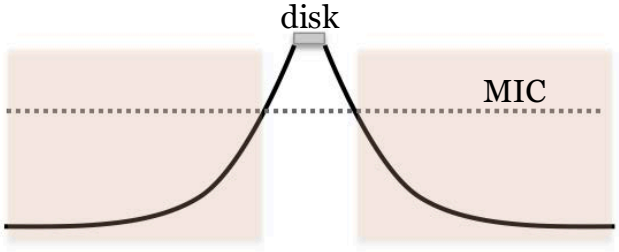
Label	Chemical	Class
A	Isopropanol	Alcohol
I	Iodine	Oxidizer
S	Dish soap	Surfactant
G	Germicidal soap	Phenolic

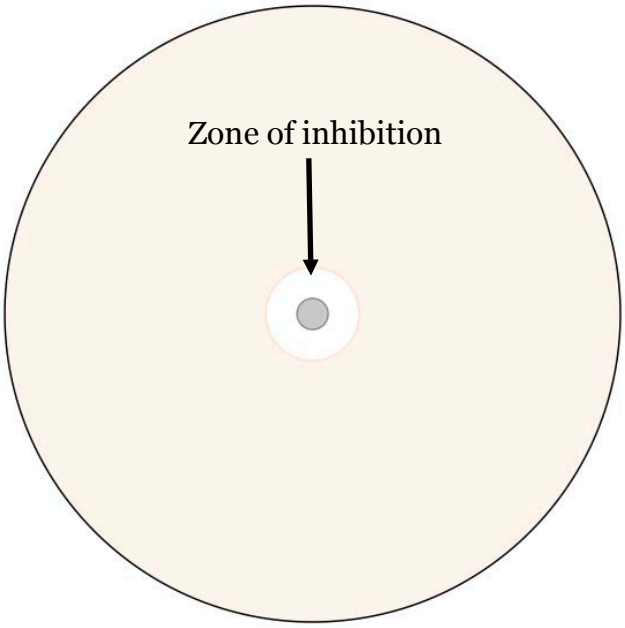
The final group of chemicals that we will employ are **antibiotics**. These compounds are produced by other microorganisms and tend to be used chemotherapeutically. The drugs that we are using today target one of the two main bacterial target structures.

Label	Antibiotic	Target
AM	Ampicillin	Peptidoglycan
CE	Cephalothin	Peptidoglycan
E	Erythromycin	70S Ribosomes
P	Penicillin	Peptidoglycan
S	Streptomycin	70S Ribosomes
TE	Tetracycline	70S Ribosomes

We will apply the compounds using paper disks and the chemical will diffuse into the media (that is why it is called the disk diffusion method!). In this process, a chemical gradient will be created. A threshold concentration (called the minimum inhibitory concentration) is necessary to kill or inhibit the microbial growth. When the drug diffuses

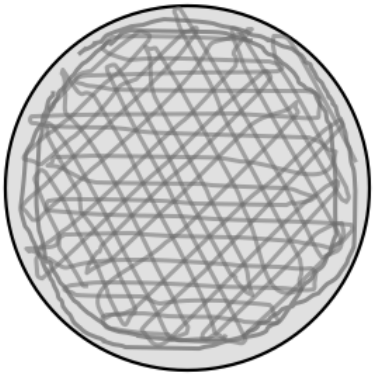
to a concentration lower than that, growth will occur. The upshot is that a **zone of inhibition** will form around our disks. Bigger zones mean that the MIC is lower (the organism is more susceptible) and the drug is more effective. Smaller zones mean that the MIC is higher (the organism is more resistant) and the drug is less effective. We will compare drug effectiveness by measuring the diameter of the zones of inhibition.





Instructions

To inoculate your plates, you will perform what I call a 3+1 streak procedure. Each group will be assigned a specific microbe to test. Label the bottoms of three petri dishes with your symbol, the organism's name, and your section. Divide two of the plates into quarters on the bottom using a sharpie. Leave the third plate undivided. Dip a sterile swab into your culture tube (or diluted culture tube for antibiotics).



- Swab the complete surface of the agar plate. Rotate the plate 120° and streak the entire surface again. Repeat once more by rotating and streaking. Finally, streak around the perimeter of the plate a couple of times. See the drawing to the right.
- Label the quarters of your plates for the antiseptics or disinfectants as shown in the introduction. Place a sterile paper disk on each quarter using a disinfected forceps. Then add one drop of the corresponding compound to each disk and allow to soak in.

• We will dilute your microbial stock for the antibiotic plate inoculation (**except for the *Candida albicans* groups – they will use undiluted microbes**). Obtain a standard tube (STD) and mix by shaking. You will use a Pasteur pipette to add you culture into a fresh tube of nutrient broth until it is approximately as cloudy as the standard (around a dozen or two drops should do it). Use the diluted microbes to do a 3 + 1 streak of your final plate.

• Use the antibiotic disk applicators to place 6 pre-loaded antibiotic disks onto your plate.

• Incubate all three plates bottoms up in a stack in the 35°C incubator.

• We will measure the diameters of the zones of inhibition to determine the relative efficacy of each compound.

• The clinical meaning of the zones of inhibition will be interpreted using the table provided below.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

• Carefully record the size of the zones of inhibition on your three plates.

You may record these data in one of two ways:

1) Photograph all three plates and tape the labeled photos in your notebook. Also complete the data table provided.
2) Carefully draw pictures of all three plates and tape the labeled photos in your notebook. Also complete the data table provided.

✪ Conclusions

1. Which disinfectant worked best?
2. Which antiseptic worked best?
3. Which was more effective, the best disinfectant or best antiseptic?
4. Which microbe was most susceptible to the disinfectants?

5. Which microbe was most susceptible to the antibiotics?
6. Which microbe was most resistant to the antibiotics?
7. Which bacterium was most susceptible to the antibiotics?

		Antiseptics				Disinfectants				Antibiotics					
	Class -->														
Organism	Compounds -->	Soap	Germicidal Soap	Isopropanol	Iodine	Bleach	Aldehyde	Lysol	Roccal	Ampicillin	Cephalathin	Erythromycin	Penicillin	Streptomycin	Tetracycline
	Candida albicans														
	Escherichia coli														
	Klebsiella pneumoniae														
	Proteus vulgaris														
	Pseudomonas aeruginosa														
	Salmonella enteriditis														
	Shigella sonnei														
	Staphylococcus aureus														

Antibiotic	Resistant	Intermediate	Susceptible
Ampicillin (*Staph*)	≤ 20 mm	21 - 28 mm	≥ 29 mm
Ampicillin (all others)	≤ 11 mm	12 -13 mm	≥ 14 mm
Penicillin (*Staph*)	≤ 20 mm	21 - 28 mm	≥ 29 mm
Penicillin (all others	≤ 11 mm	12 - 21 mm	≥ 22 mm
Cephalothin	≤ 14 mm	15 - 17 mm	≥ 18 mm
Erythromycin	≤ 13 mm	14 -17 mm	≥ 18 mm
Streptomycin	≤ 11 mm	12 - 14 mm	≥ 15 mm
Tetracycline	≤ 14 mm	15 -18 mm	≥ 19 mm

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your Pasteur pipettes in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Microbial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Candida albicans (BSL-1) Candida albicans* (BSL-1) This yeast is a member of our body's normal microbiota.
2. *Escherichia coli (BSL-1). The K12 strain is the most studied organism on the planet.*
3. *Klebsiella pneumoniae* (BSL-2) Strains of this microbe can cause pneumoniae in kids.
4. *Proteus vulgaris* (BSL-2). This microbe is commonly found in soil and water. This bacterium can sometimes cause urinary tract infections.
5. *Pseudomonas aeruginosa (*BSL-2) This is commonly found in wet environments and can be a potent pathogen under the right circumstances.
6. *Salmonella enteritidis* (BSL-2) This microbe is associated with food-borne infections.
7. *Shigella sonnei* (BSL-2) This microbe is also associated with food-borne infections.
8. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed your stains.

Contaminated materials

Dispose of your used cotton swabs in the orange bag-lined silver cans when you have completed your inoculations. Your petri dishes should go there too when you have completed your observations.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial

culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

18

Thermal Death Curves

Background

Microbes differ in their sensitivity to heat. Treated long enough at a high enough temperature, bacterial cells (and even endospores) will die. The **D-value** represents the conditions – time, temperature, and other parameters – that results in a reduction of the bacterial population by 90%. That is, only 10% (one log) of the viable cells remain. Many applied microbiologists want to sterilize objects. To do so, they must treat the object such that enough D-values have elapsed to ensure that all cells have been killed. There are two ways to measure this: the thermal death time and the thermal death point.

The **thermal death time** is measured while holding temperature constant. The question answered is, "how long must we heat at this temperature to kill all viable cells?"

The **thermal death point** is measured while holding time constant. The question answered is, "how hot must we heat for this length of time to kill all viable cells?"

Today, we will measure the thermal death time and thermal death point for three different bacterial cultures.

Instructions

- We will work in 15 groups 1 or 2 people per group . Each group will be responsible for sampling one of our microbes at one of the temperatures.
- Every group will need 5 empty petri dishes. Label the bottoms (the smaller diameter piece) with your section number and the organism name using a sharpie. Label each plate for one of our time points 0, 1, 2, 5, and 10 minutes . Transfer 0.1 ml of your culture into the bottom of the empty 0 minute plate. Add growth media to your sample by pouring in a molten 12 ml pour tube, mix by slowly swirling, and cover.
- Next, put your bacterial culture tube into the water bath at your specified temperature. After one minute elapses, withdraw a 0.1 ml aliquot and deliver it to the bottom of the 1

minute plate. Add media, swirl, and cover. Repeat this process at 2 minutes, 5 minutes, and 10 minutes.

- After all of your plates have hardened 10 minutes , place them as a stack – bottoms up – in the 30°C incubator. We will evaluate the microbial growth next time.

Notebook Entry

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✪ Observations

• Record the growth of your organism for each of your five plates. I suggest that you rate a plate with 0 to 10 colonies as a (–) and those with 11 or more colonies a (+).

• Obtain the other growth scores from your classmates.

• You may record these data using the following tables and taping them into your notebook. Alternatively, you can create your own tables in your notebook.

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the

	Time at temperature					
Temperature	0 min	1 min	2 min	5 min	10 min	TDT
60°C						
70°C						
80°C						
90°C						
100°C						

	Time at temperature					
Temperature	**0 min**	**1 min**	**2 min**	**5 min**	**10 min**	**TDT**
60°C						
70°C						
80°C						
90°C						
100°C						

	Time at temperature					
Temperature	**0 min**	**1 min**	**2 min**	**5 min**	**10 min**	**TDT**
60°C						
70°C						
80°C						
90°C						
100°C						

✪ Conclusions

1. What were the thermal death times for each organism?
2. What were the thermal death points for each organism at 5 minutes?
3. Which microbe had the highest thermal death point?
4. Speculate upon why the organism in question three might be heat tolerant.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your used pipettes in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash. Dispose of your empty tubes in the silver cans that do not have an orange bag.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Bacillus megaterium* (BSL-1). This is yet another common endospore-forming soil bacterium.
2. *Escherichia coli* (BSL-1). The K12 strain is the most studied organism on the planet.
3. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Dispose of your bacterial strains in the unlined silver cans at the end of your heat treatments.

Contaminated materials

If your spill your culture, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets. When you are finished observing your plates, they should be placed in the silver cans lined with an orange biohazard bag.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

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this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

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19

Water Quality Testing

Background

Many important microbial diseases are transmitted via the fecal-oral route. In this mechanism, food or water supplies are contaminated with fecal material of either human or animal origins and directly consumed by people. Hundreds of different pathogens (including helminths, protozoa, bacteria, and viruses) can potentially be present in surface waters. Directly testing for each of these microbes is not feasible – there are just too many possibilities. In addition, many of these are difficult and/or dangerous to cultivate and identify in the laboratory. As an alternative, microbiologists typically use "**indicator organisms**" to detect possible fecal contamination.

A good indicator organism should have several properties:

1. The organism should always be present when feces are present.
2. The organism should be absent when feces are absent.
3. The organism should be very easy to grow.
4. The organism should be easy to identify (using differential media).
5. The organism should persist in the environment slightly longer than the pathogens (there is less problem with false positives than false negatives).

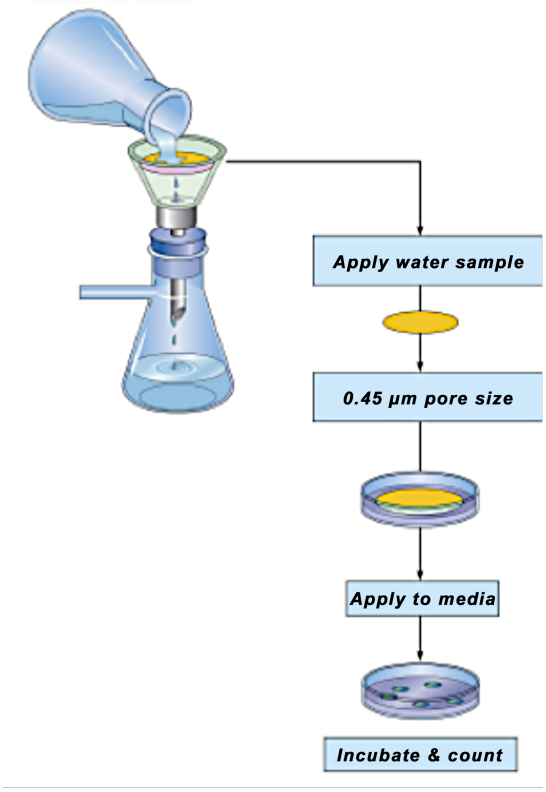
The most common indicators used to detect fecal contamination are fecal coliforms and fecal streptococci. **Fecal coliforms** are lactose-fermenting gamma proteobacteria (like *Escherichia coli*). Coliforms are commonly found in mammalian feces – including humans – and are considered good proxies for detecting potential human fecal contamination. The Department of Environmental Quality has empirically determined coliform levels that correspond to water safety. Our wastewater treatment plant is not allowed to discharge more than 400 fecal coliform cfu/100 ml into our river. The river itself must have fewer than 160 fecal coliform cfu/100 ml to be considered safe for recreational purposes. Drinking water (having the most rigorous standards) must have <1 fecal coliform cfu/100 ml. Knowing that the water is safe or not is good. However, we sometimes would also like

to make inferences about the probable source of contamination. That is what the other indicator organism is good for.

Fecal streptococci – like *Enterococcus faecalis* – are common in the feces of animals (like ducks) but not humans. By comparing the ratio of coliforms to streptococci, we can make an informed guess as to where the fecal contamination originated. The ratios that we will be using are indicated to the right.

Ratio	Probable Source
Blue/Red ≥ 4:1	Human
2:1 ≥ Blue/Red < 4:1	Mixed
0.7:1 ≥ Blue/Red < 2:1	Unknown
Blue/Red < 0.7	Animal

Instructions



We will be evaluating water samples from three different locations at two different dilutions in duplicate (12 stations). The class will divide into twelve pairs – each pair claiming one sample for processing. Each pair will need one plate of two different media: mFC and mEA. The **mFC** plate is used to detect fecal coliforms. It contains lactose and resorcinol dye. These components make fecal coliform colonies appear blue after incubation. The media also contains bile salts that inhibit the growth of non-coliform bacteria. We will incubate these plates at 44.5°C to prevent the confounding by non-fecal coliforms; environmental coliforms cannot ferment lactose at elevated temperatures. The **mEA** plate is used to detect fecal streptococci. This media contains the metabolic poison sodium azide. The media also contains tetrazolium red. Any azide-resistant bacteria will grow and turn red as the reduce the tetrazolium dye. The water sample discussed in the introduction are expressed in cfu/100 ml. Our plates are too small to plate that much water! Therefore, we will concentrate our samples by filtration. We will apply 20 ml of your sample to a filter with a pore size of 0.45 µm. That is too small for bacteria to pass through. Once your sample has filtered, we will apply an 80 ml sterile water rinse to remove any inhibitory compounds from the water sample. The filter will be placed grid-side-up onto the surface of the media. After incubation, we will count the

number of colonies and calculate both fecal coliforms per 100 ml and the ratios of coliforms to streptococci. The dilution calculation is similar to what we've done before.

$$\frac{cfu}{100ml} = \frac{cfu \bullet SDF}{volume\ plated} \bullet 100$$

Notebook Entry

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✪ Observations

Sample	Dilution	mFC	FC / 100mL	mEA	FS / 100 mL
Clear Lake	10^0				
	10^1				
Lake Andrews	10^0				
	10^1				
Mitchell Creek	10^0				
	10^1				

• Complete the table to the right with regard to the media used in this exercise.

	mFC (blue juice)	mEA (tan)
Indicator strain		
Selective		
Differential		
Colony color		
Incubation temperature		

✪ Conclusions

1. Which of these bodies of water (if any) is safe for recreational use?
2. Which of these bodies of water (if any) is safe for drinking?
3. What is the most probable source of contamination for each of these bodies of water?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Contaminated materials

When you have completed your observations, you should discard your mFC and mEA plates in the orange bag-lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

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Hand washing

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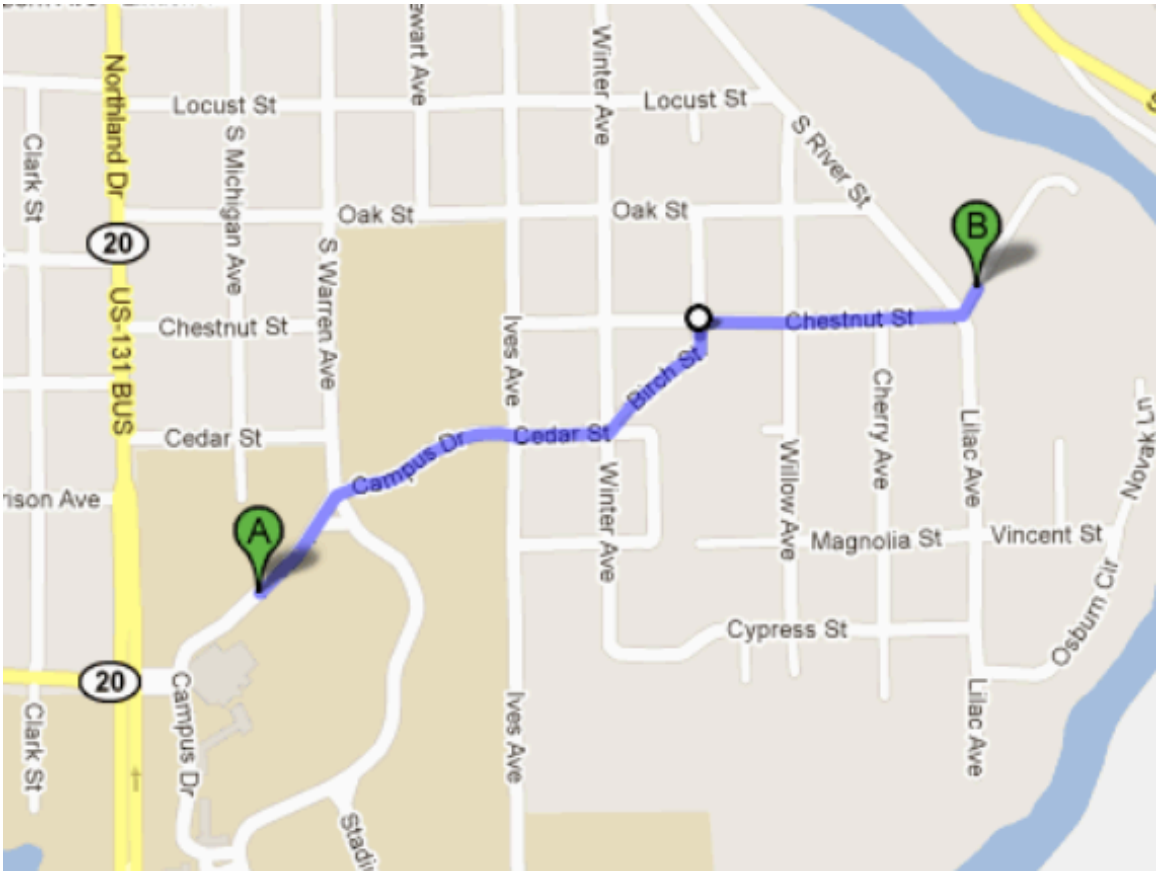
20

Wastewater Treatment

Background

For this exercise, we will be touring the Big Rapids wastewater treatment plant. We will meet at the plant. You may follow these directions the plant – it is by the river near the dog park.



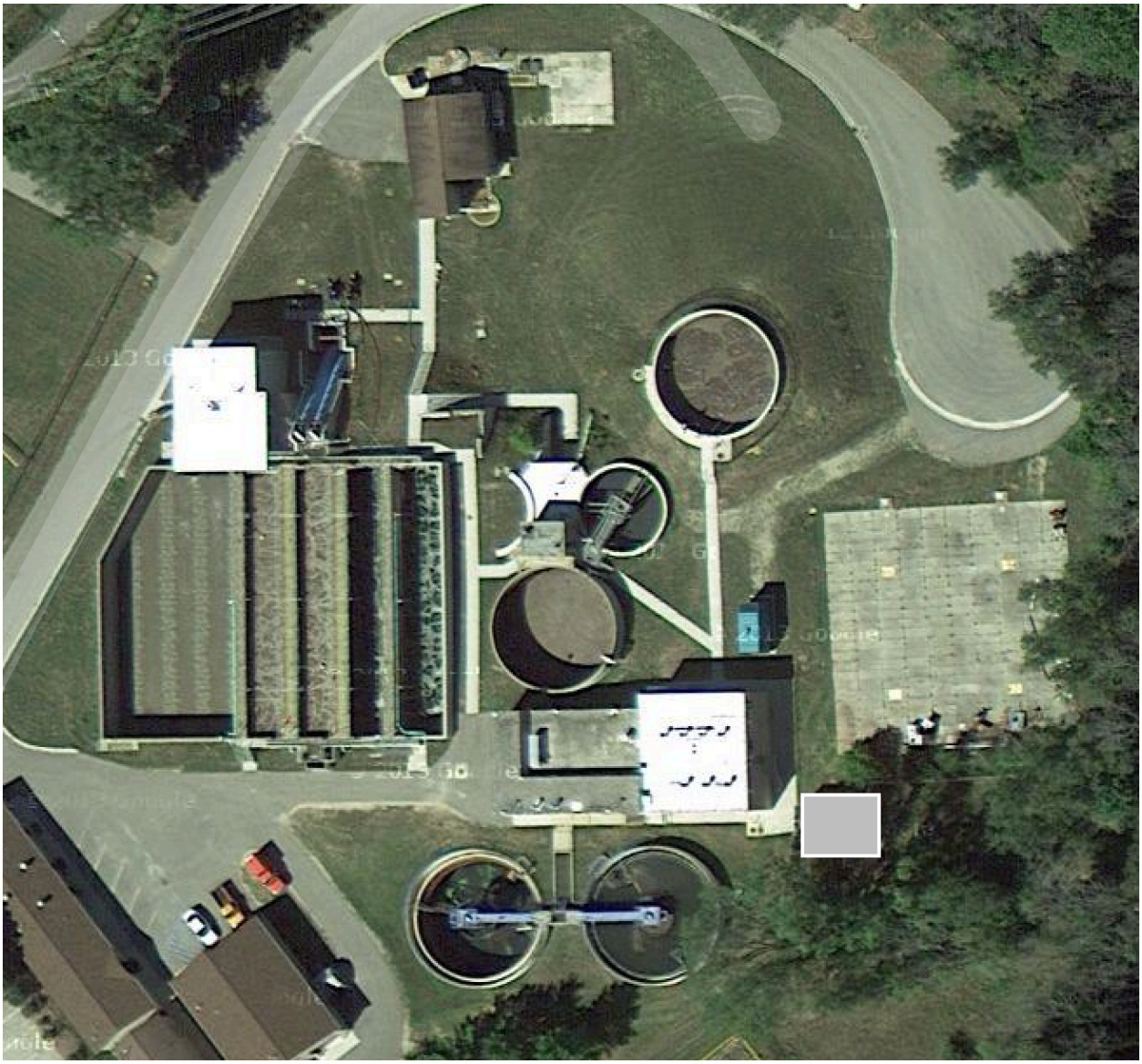
The wastewater plant performs three main functions:

1) Physical removal of large and undesirable objects.
2) Biological and/or chemical removal of excess organic material.
3) Disinfection to reduce the number of potential pathogens.

Instructions

Attend the tour of the wastewater facilities and identify the locations and primary functions of the following :

Head works, screw pumps, grit and grease removal, aeration basins, equalization basin, FeCl3 addition, clarifiers, UV irradiation, sludge thickener, aerobic digesters, sludge storage. (one building was blocked by foliage – I indicated its position with a gray box).



Notebook Entry

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✪ Observations

Clearly indicate the positions and functions of each of the items mentioned in the introduction.

✪ Conclusions

1. On average, about how many gallons of wastewater are treated here each day?
2. What is the dissolved oxygen level in the operating aeration basins? Why are these numbers different from each other?
3. What were the most recent numbers of fecal coliforms per 100 ml for the plant? How does this number compare with your previous mFC counts for the river?
4. Why shouldn't you dump grease down your sink?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

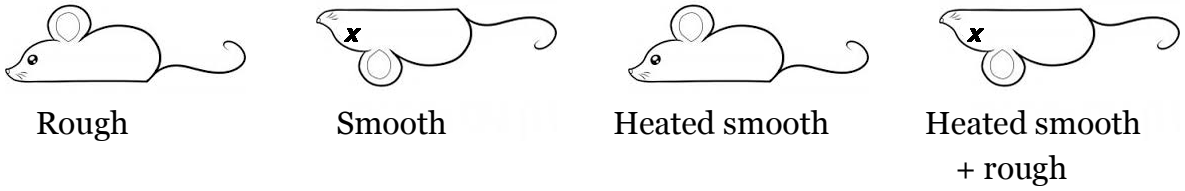
You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab. **THIS SITE TREATS HUMAN WASTE THAT MAY CONTAIN VIABLE PATHOGENS! WASH YOUR HANDS!!**

21

Genetic Transformation

Background

Bacteria can horizontally transfer genes (within the parental generation) in at least three different ways – transformation, conjugation, and transduction. Genetic transformation was the first of these to be described and is still an important tool in recombinant DNA laboratories. This process was first described by Frederic Griffith in the 1920s. He was working with two strains of *Streptococcus pneumoniae* – a "rough" strain that made small compact colonies on lab media and a "smooth" strain that made larger mucoid colonies. When injected into mice, the following results were observed.



Rough | Smooth | Heated smooth | Heated smooth + rough

The rough strain was avirulent to when injected into mice. The smooth strain, however, efficiently killed mice and could be isolated from the mice postmortem (Koch's postulates). Heat killing the smooth strain made them non-toxic (no real surprise there…). The interesting thing was this: mixing the dead smooth strain with the live rough strain killed the injected mice. Neither of these were virulent when injected individually. Even more strange – live *smooth* bacteria were isolated from the dead mice. Something had **transformed** the rough strain into a smooth strain. Oswald Avery proved that the transforming material was DNA in the 1940's.

It turns out the some bacteria are capable of transporting exogenous DNA into the cell and incorporating it into their genomes. Such cells are called **competent**. *Streptococcus pneumoniae* happens to be naturally competent. Other bacteria, like *Escherichia coli*, are not naturally competent but can be made competent artificially in the laboratory. The point of today's exercise is to explore competence with a different bacterium: *Acinetobacter calcoaceticus*.

Instructions

We will be using two strains of *Acinetobacter calcoaceticus* in this exercise: one is susceptible to the antibiotic streptomycin while the other is resistant. These correspond to Griffith's rough and smooth strains, respectively.

Day one:

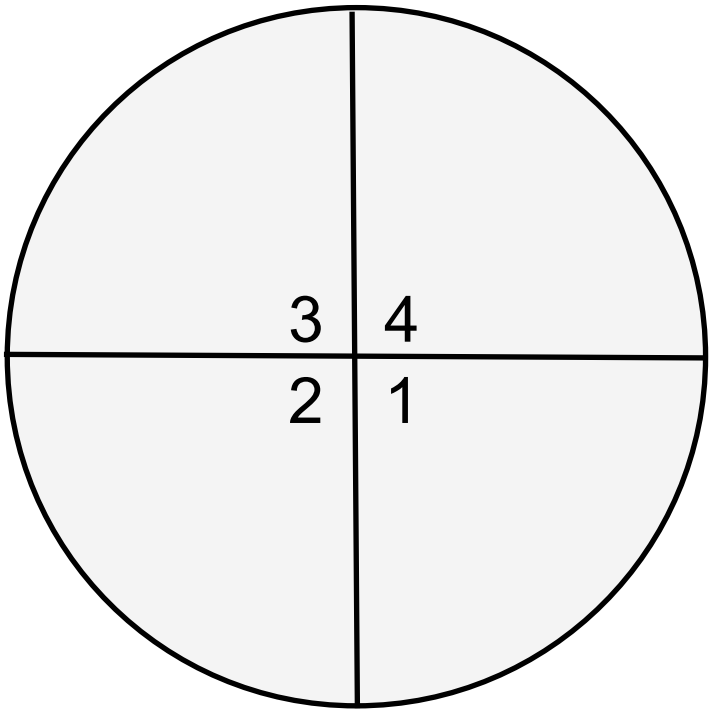
You will first need to heat kill some of the resistant strain.

- Get a tube of saline citrate SDS buffer and label the cap with a unique symbol.
- Flame a loop and add a loopful of the resistant strain to the buffer.
- Place your tightly capped tube in a 60°C water bath for at least 45 minutes.

The combination of detergent (SDS), osmolarity, and temperature will efficiently kill and lyse the resistant cells – releasing their DNA genomes into the solution. After your heat treatment is complete, recover your tube of buffer.

Obtain a plate of nutrient agar and label the bottom with your initial and section number. Then divide the bottom into quarters as indicated below.

- Flame your loop and let it cool. Then streak two loopfuls of the heat-killed resistant strain into each of the "3" and "4" quadrants. Flame and cool your loop between each transfer.
- Flame your loop again and let it cool. Streak the live resistant strain in quarter "1".
- Flame your loop again and let it cool. Now streak the live susceptible strain in each of the "2" and "4" quarters. Flame and cool your loop between each transfer.



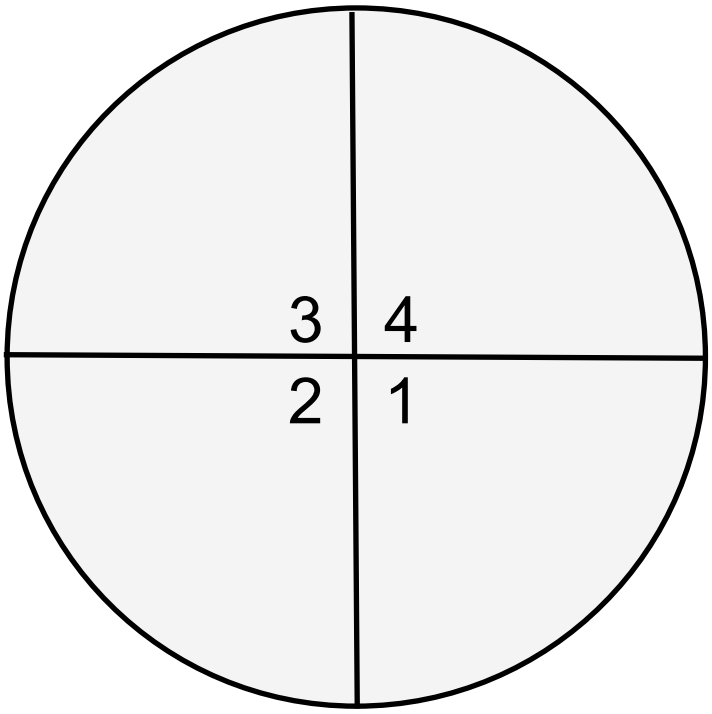
Incubate your plate, bottoms-up, in the 35°C incubator.

Day two:

Observe the growth (or lack of it) in the four quarters of your plate. Obtain another plate of nutrient agar. This time, your plates will contain the antibiotic streptomycin. Label these plates exactly the same way as before.

- Flame your loop and let it cool. Then streak a small quantity of the growth from quarter "1" of your original plate into quarter "1" of the streptomycin plate.
- Repeat this process for the other three quadrants.

Incubate your plate, bottoms-up, in the 35°C incubator.



Day three:

Observe the growth (or lack of it) in the four quarters of your plate.

Notebook Entry

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✪ Observations

• Carefully record the growth on all four quarters of each of your plates. Indicate clearly which plate is which!

You may record these data in one of two ways:

1) Carefully sketch your observations by hand and label each plate and quarter.

2) Photograph each plate, print the pictures and tape them into your notebook. Once again, be sure to clearly label your photos.

✪ Conclusions

1. For each quarter (1-4) of the nutrient agar plate, explain why bacterial growth was or was not present.
2. For each quarter (1-4) of the streptomycin agar plate, explain why bacterial growth was or was not present.
3. Which step of Griffith's transformation experiment corresponds to each of our plate quarters?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Acinetobacter calcoaceticus* (BSL-2). This microbe is commonly found in soil and is also present in the gut contents of humans and some mosquitoes.

Return the cultures when you have completed your inoculations.

Contaminated materials

Your tubes of heat killed bacteria should be disposed of in the unlined silver buckets. When you have finished observing your plates, they should be discarded in the orange bag lined silver buckets. If you spill any of the cultures today, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

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Hand washing

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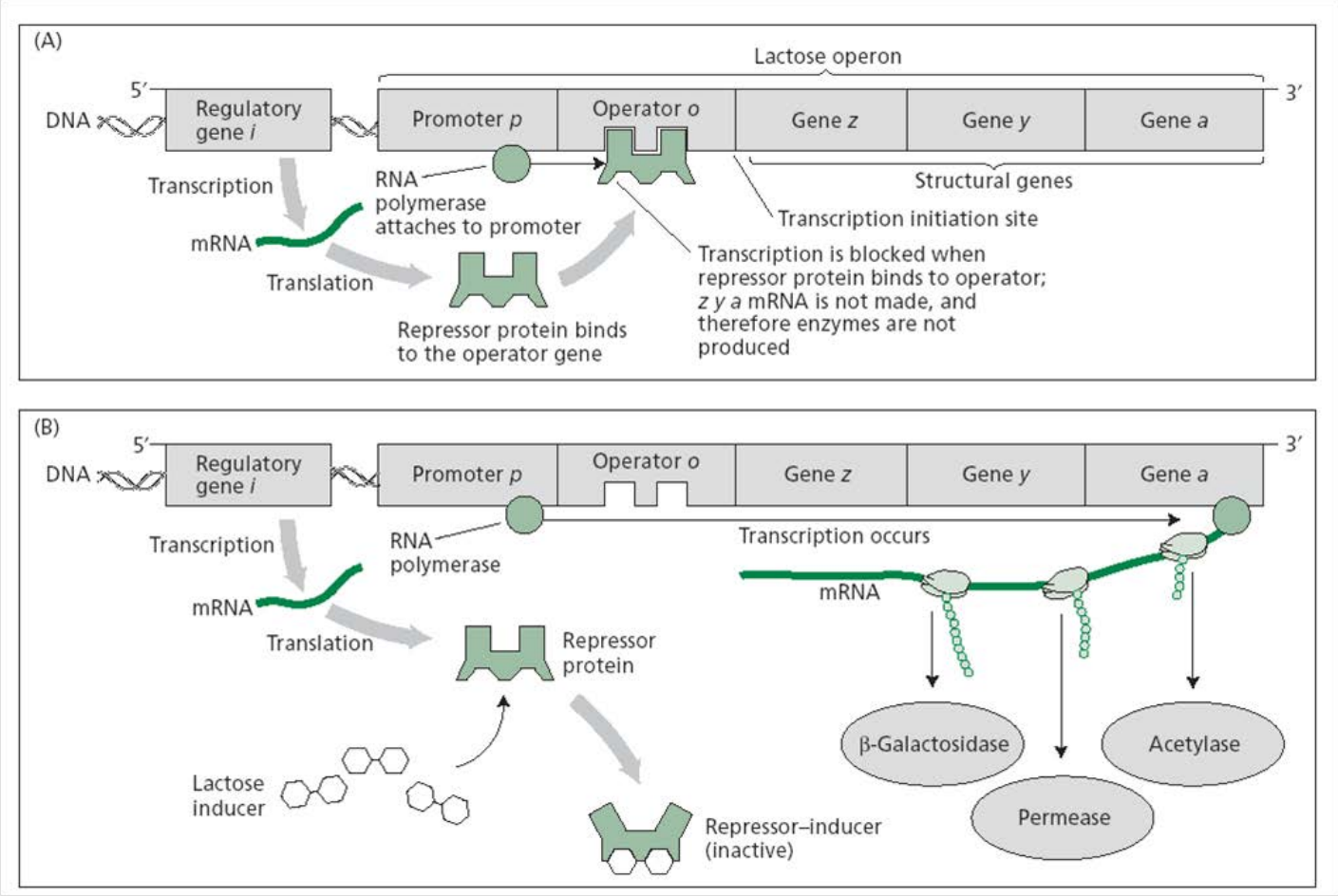
22

Lactose Operon Regulation

Background

The *lac* operon encodes three proteins: β-galactosidase (the product of the *lac*Z *gene*), lactose permease (the product of the *lac*Y gene), and lactose transacetylase (the product of the *lac*A gene). The function of *lac*A is not known, but a mutation in either *lac*Z or *lac*Y means that the cell can't grow by using lactose as a sole carbon source. All three structural genes are transcribed from a common promoter site, in the direction Z → Y → A. The *lac* transcript is termed **polycistronic** because it contains more than one coding sequence.

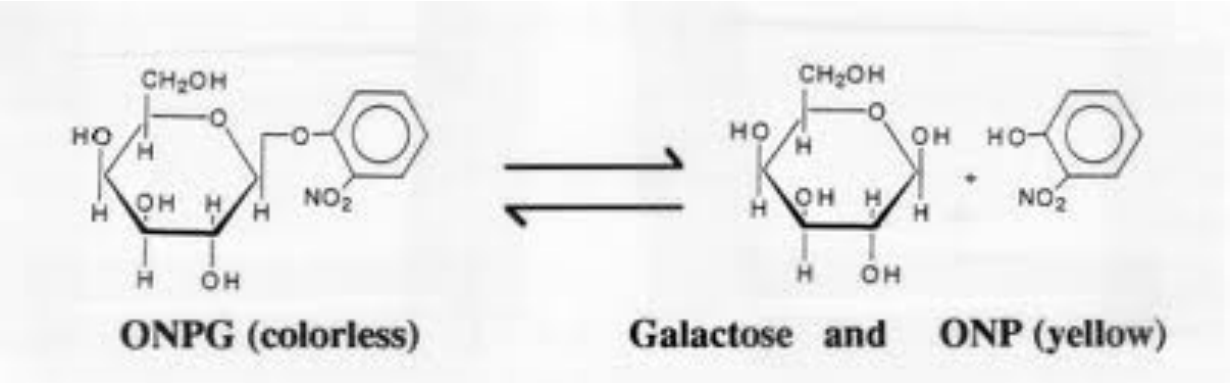
Closely linked to the *lac* structural genes is the gene (*lac*I) for the *lac* **repressor**, a tetramer of four identical subunits. The repressor has two functions. First, it binds to the DNA near



the *lac* promoter and prevents transcription of the structural genes. Secondly, it binds to a small molecule called an **inducer**. In the cell, the inducer is allolactose, a metabolite of lactose. The binding of inducer to the repressor is **cooperative**, meaning that the binding of one molecule of inducer makes binding of the next one more favorable, and so on. This means that the repressor binds inducer in an all or none fashion.

In the absence of inducer, the repressor protein binds to a sequence called the **operator** (*lac*O) which partially overlaps with the promoter. When it is bound to the operator, *lac* repressor allows RNA polymerase to bind the promoter and form an open complex but not to elongate transcription. Repressor is therefore a negative regulator of gene expression: If repressor is not present (for example, the bacterium is deleted for the *lac*I gene), then transcription of the *lac* genes occurs, and the structural genes are expressed whether or not inducer is present. The unregulated, "always on" expression caused by a *lac*I $^{-}$ mutation is called **constitutive expression**. This behavior is characteristic of a negative control element. Other mutations of *lacI* disrupt its ability to bind the inducer. These proteins (termed *lacI*^{s}) never leave the operator and result in **repressed expression**. *Lac*O is another negative element. Deletion of *lac*O leads to constitutive expression of the *lac* genes. Mutation of *lacO*, that result in loss of repressor binding (called *lacO*^{c}*)* also result in constitutive expression. The difference between these mutation can be seen when the genes are put into an artificial situation where two copies of the relevant control genes exist (this condition is called a **merozygote**).

Jacob and Monod used the fertility plasmid (F) from *Escherichia coli* to study the regulation of the lactose operon. They used a variant of this plasmid (called F') that contained a normal functional copy of the lactose operon genes. For our purposes, we will assume that the F' contains a functional LacI and LacZ protein and that the LacZ gene on the F' contains wild type operator and promoter sequences. Five strains (wild type, LacI-, LacZ- LacIs, and LacOc) will be considered in this exercise. These strains were grown in the presence of glucose (G) or lactose (L). In addition, the F' construct was introduced into the strains by conjugation and these strains were also grown in the presence of glucose or lactose. Enzyme activity of LacZ (beta galactosidase) in these four cultures was measured using ONPG. This is a colorless chemical analog of lactose. When cleaved by LacZ, ortho-nitrophenol (yellow in color) is produced. Therefore, if the cultures turn yellow, the lactose operon (LacZ at least) is being expressed. If LacZ is not expressed or functional, the cultures remain colorless.



Instructions

Based upon what you have learned so far, complete the worksheet for the five strains.

Notebook Entry

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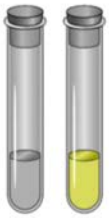
✪ Observations and Conclusions

• Carefully record the LacZ activity of all 10 culture tubes. The best way to do this is to cut out the pictures from your work sheet and tape them into your notebook. For each of the five strains, indicate the phenotype observed for the mutant and merozygote, the genotype if the mutants and provide a brief explanation (in your own words) of what is going on in these tubes.

Strain A

Strain A
F′ I⁺O⁺Z⁺



Phenotype (constitutive, repressed, inducible)
Strain: _____
Strain + F′: _____
Circle the identify of the strain:
[I⁻ Iˢ Oᶜ Z⁻ WT]
Explain what is going on in a sentence or two:
_____
_____
_____
_____

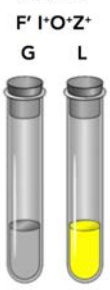
Strain B

Strain B
F′ I⁺O⁺Z⁺

Phenotype (constitutive, repressed, inducible)
Strain: _____
Strain + F′: _____
Circle the identify of the strain:
[I⁻ Iˢ Oᶜ Z⁻ WT]
Explain what is going on in a sentence or two:
_____
_____
_____
_____

Strain C

Strain C
F′ I⁺O⁺Z⁺



Phenotype (constitutive, repressed, inducible)
Strain: _____
Strain + F′: _____
Circle the identify of the strain:
[I⁻ Iˢ Oᶜ Z⁻ WT]
Explain what is going on in a sentence or two:
_____
_____
_____
_____

Strain D

Strain D
F′ I⁺O⁺Z⁺

Phenotype (constitutive, repressed, inducible)
Strain: _____
Strain + F′: _____
Circle the identify of the strain:
[I⁻ Iˢ Oᶜ Z⁻ WT]
Explain what is going on in a sentence or two:
_____
_____
_____
_____

Strain E

Strain E
F′ I⁺O⁺Z⁺

Phenotype (constitutive, repressed, inducible)
Strain: _____
Strain + F′: _____
Circle the identify of the strain:
[I⁻ Iˢ Oᶜ Z⁻ WT]
Explain what is going on in a sentence or two:
_____
_____
_____
_____

Housekeeping

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Laboratory bench

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Cell phone / camera

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Hand washing

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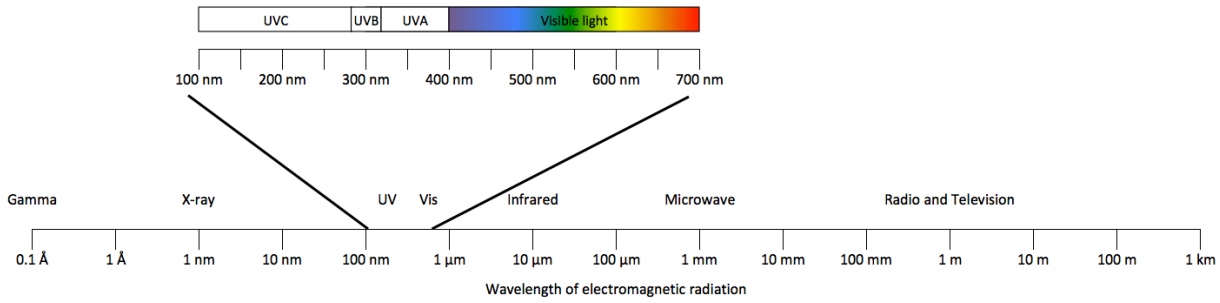
23

Ultraviolet Light

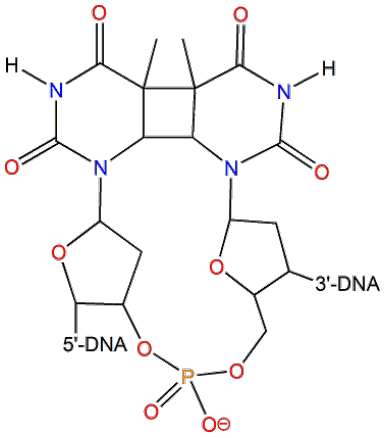
Background

The electromagnetic spectrum consists of radiation ranging from very short to very long wavelengths (see the illustration below). In general, shorter wavelength radiation has more energy, has a higher frequency, has greater penetrating power, and is more biologically damaging than longer wavelength radiation. We are most familiar with visible light (wavelength 400 to 700 nm). Ultraviolet light has a shorter wavelength than visible light. As a result, UV light has more energy than visible light and can be used as a germicidal treatment.



Ultraviolet radiation is broken into three sub-spectra (A, B, and C). UVA has wavelengths between 315 and 400 nm. These are the wavelengths that give you a suntan and are the least damaging. UVA is partially removed from the solar spectrum by the ozone in our atmosphere. UVB has wavelengths from 280 to 315 nm. These wavelengths are more damaging and cause sunburns and skin cancer. Most, but not all, of the UVB portion of the solar radiation is removed by ozone. The shortest wavelength UV light (UVC; 100 to 280 nm) is the most damaging. These wavelengths are completely removed from sunlight by ozone in our atmosphere. We can, however, produce these wavelengths using special bulbs – like the small ones we use in this lab or the larger ones that you will see at the wastewater treatment plant later.

Ultraviolet light is most germicidal around 260 nm. DNA strongly absorbs energy at this frequency and that leads to DNA damage. One common form of DNA damage is the formation of pyrimidine dimers (see picture to the right). The extra bonds between the pyrimidine bases prevents proper base pairing in the double stranded DNA. The repair process involved in resolving this situation is error-prone. Errors due to UV exposure result in mutations of the DNA sequence. If enough mutations accumulate, the cell will die. We will compare the relative susceptibilities of four bacteria to UV exposure today.



Instructions

Working in groups, we will expose four different microbes to different amounts of UV light. Each group should have a tube of one of the organisms, a UV light, an exposure template, 8 small petri dishes of media, and 8 sterile cotton swabs.

We will perform the exposures under subdued lighting. Some strains of bacteria can perform photo-repair (they have a DNA repair enzyme that uses visible light to repair UV damage).

- Plug in your UV light and turn the power on. DO NOT LOOK DIRECTLY AT THE LIT BULB; your eyes do not like UV light. Let the lamp warm up for at least five minutes.
- Label the bottom of each plate with the name of your organism, you group's identification, and the eight time points (0, 5, 10, 20, 30, 50, 70, and 90 seconds).
- Dip a cotton swab in your culture and inoculate the surface of your agar media using a 3 + 1 streaking technique (repeat for all eight plates).
- Set the 0 seconds (unexposed plate aside for now).
- Place the 5 second plate right-side-up in the smaller circle on your exposure template.
- Remove the cover from the 5 second plate and place the UV light in the larger circle on your exposure template.
- After 5 seconds, remove the lamp and replace the cover on the 5 second plate. Set it aside with the unexposed plate.
- Repeat this process for the other plates adjusting the exposure time as appropriate.
- When you are finished, place all of your plates bottoms up as a stack in the 20°C (room temperature) incubator.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

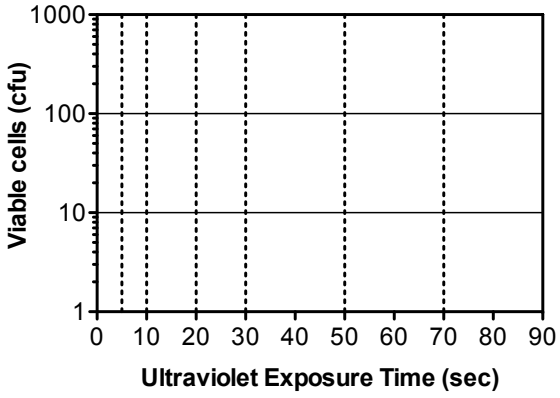
✪ Observations

• To the best of your ability, determine the number of CFU on each of your 8 plates.

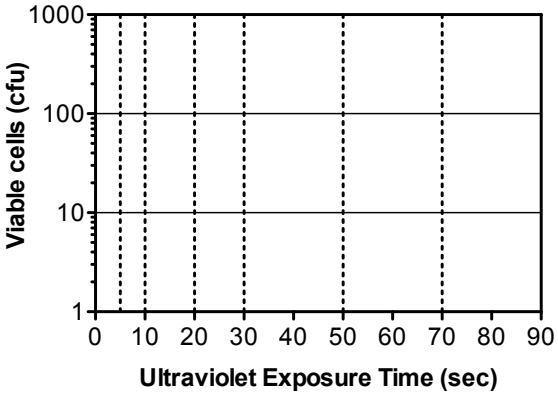
• Plot your data points on the semi-log plots provided (get the other group's data to complete your entry.

Effect of Ultraviolet Light

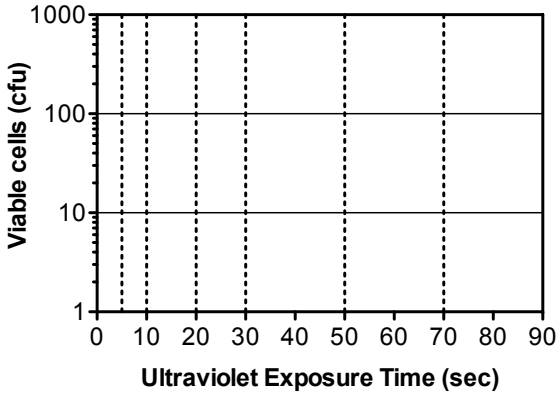
Bacillus cereus



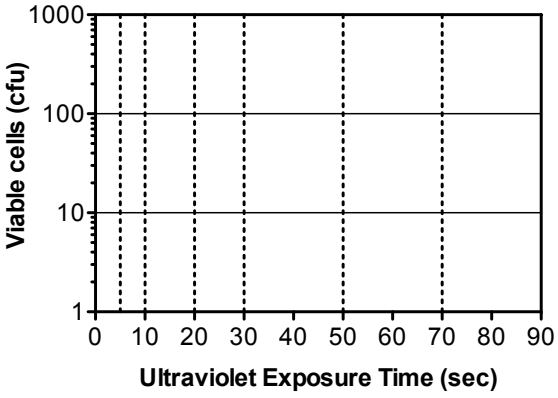
Pseudomonas aeruginosa



Serratia marcescens



Staphylococcus aureus



✪ Conclusions

1. Which microbe was most resistant to UV light killing?
2. What accounts for this resistance?
3. Predict what would have happened if we left the covers on the petri dishes while the cells were exposed to UV light.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Ultraviolet lamps

When you are finished, wrap up the power cord around the neck of the UV lamps and return them to the front of the lab.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Bacillus cereus (BSL-1).* This is a common endospore-forming bacterium from the soil.
2. *Serratia marcescens* (BSL-1) This gamma proteobacteria produces a red pigment and is very rarely pathogenic.
3. *Pseudomonas aeruginosa (*BSL-2) This is commonly found in wet environments and can be a potent pathogen under the right circumstances.
4. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.

Return the bacterial culture when you have completed your stains.

Contaminated materials

Dispose of your used cotton swabs in the silver cans lined with an orange biohazard bag. At the end of this exercise, you should dispose of your petri dishes in the orange bags as well. If you spill a culture, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

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Hand washing

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24

Microbial Symbioses

Background

Most microorganisms live together in interacting communities in the environment. These inter-microbial relationships form the base of the (and sometimes the entire) food web. Microbes can exhibit any and all of the possible relationships with other microbes. Some common relationships that we have seen (and you should know) from the lab are:

Competition: A contest between organisms for territory, a niche, or resources. Soil bacteria are often in direct competition for carbon sources (such as starch) in their environment. In this case, neither organism fully thrives – both are harmed somewhat.

Cooperation: The process of groups of microbes working or acting together for their common/mutual benefit. An example of this is quorum sensing (talked about in regulation in our lectures). Both organisms can benefit from this interaction.

Predation: An interaction where a predator (an organism that is hunting) feeds on its prey (the organism that is attacked). Predators tend to be as large or larger than their prey. An example is *Paramecium* consuming bacteria. Obviously, the predator benefits, but the prey…. Not so much.

Mutualism: A relationship two organisms of different in which each individual benefits from the activity of the other. This is exemplified by lichen, ruminants, and alfalfa.

Commensalism: A class of relationships between two organisms where one organism benefits without affecting the other. Many members of your microbiota are commensal – they benefit from you but not vice versa.

Amensalism: An interaction where an organism inflicts harm to another organism without any costs or benefits received by the actor. An example of this is antibiotic production by a fungus.

Parasitism: An interaction where one species, the parasite, benefits at the expense of the other, the host. Parasites tend to be smaller than their hosts. An example of a parasite is bacteriophage T4 (all viruses are considered to be obligate intracellular parasites).

In this exercise, you will be observing examples of mutualism and commensalism. You have seen examples of parasitism and amensalism in previous exercises.

Instructions

Day 1: While working in pairs, you should set up three nutrient broth tubes as follows:

- Label the tubes 1, 2, and 3 and put a symbol on each of the caps to make it easier to find them again.
- Flame a loop and let it cool. Then use the loop to add a small amount of *Staphylococcus aureus* (from the plate provided) to tube number 1.
- Repeat the prior step to inoculate tube number 3 with *S. aureus* as well.
- Using a sterile Pasteur pipette, add three drops of the *Clostridium sporogenes* broth culture to tubes number 2 and 3.
- Loosely cap all three tubes and incubate at 35°C.

Day 2: Observe the growth in your three tubes.

- Record your observations in your notebook.
- Gram stain the cultures from tubes 1 and 3.
- Use about 3 loopfuls of each on separate slides. Let this air dry and then heat fix.
- Record your observations in your notebook

In addition, you should observe the examples of lichen displayed in our lab. These lichen are actually communities of fungi and algae.

Finally, you will need to prepare a wet mount of termite hindgut contents.

- Put a drop of water on a microscope slide.
- Capture a termite and place it in the drop of water.
- Use a coverslip to squash the termite. You will thereby release the hindgut contents (bacteria and protozoa) for viewing.
- View using your high dry objective (you will want to reduce the brightness of the light using the iris in the substage condenser).
- Try to find ciliate protozoa and spiral-shaped bacteria. These are actually the microbes responsible for cellulose degradation in the termite.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

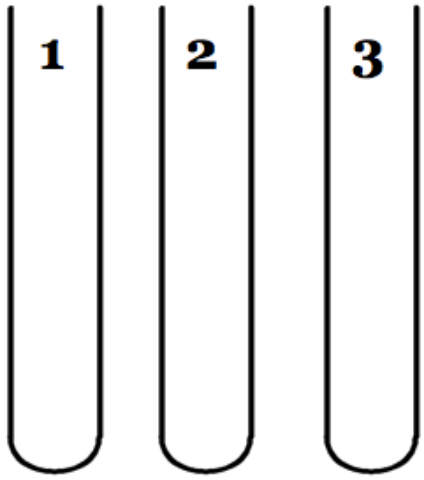
✪ Observations

• Clearly record the appearance of your broth tubes. You can draw them using the following diagrams or photograph them and tape the picture into your notebook.

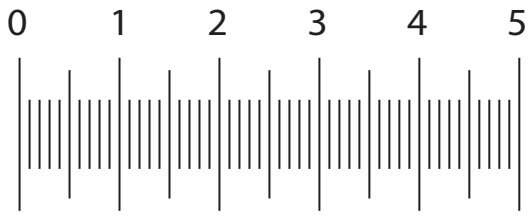
• Carefully record the size, shape, arrangement, and color of the cells in tubes 1 and 3. Search around your slides and make sure that you see all types of cells present.

You may record these data in one of two ways:

1) Record the view of the ocular micrometers and bacterial cells in focus for each of the eight different samples. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well. Also ensure that your drawings show the correct size, shape, arrangement, and color of each cell type.

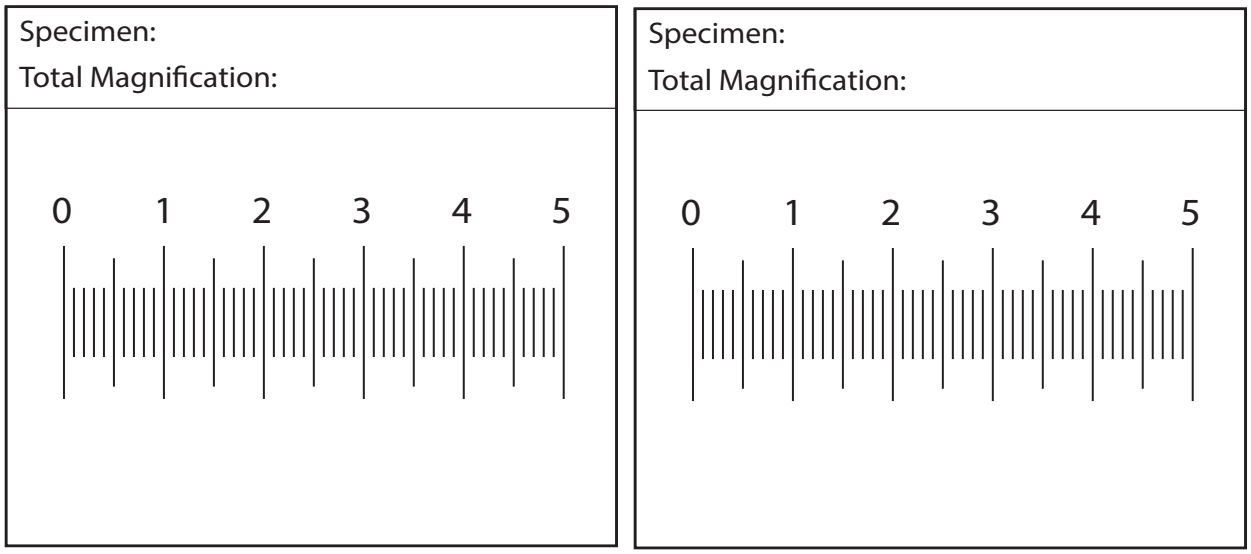


Specimen:
Total Magnification:



Specimen:
Total Magnification:

You should also include a picture or drawing of the termite hindgut contents and the lichens that you observed.



✪ Conclusions

1. Why was there no growth in tube 2?
2. What sort of relationship is illustrated in tube 3?
3. Which microbe benefitted from the relationship in tube 3?
4. How does the result in tube three relate to gingivitis (gum disease)?
5. What would happen to the termites if all its hindgut microbes were eliminated? Why?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your Pasteur pipettes, glass slides and coverslips in the glass waste box when you are finished for the day. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.
2. *Clostridium sporogenes* (BSL-1) This is basically *Clostridium botulinum* without toxins.

Return the bacterial culture when you have completed your stains.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

Dispose of your three broth tubes in the unlined silver buckets when you have completed your observations and stains. If you spill any cultures, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

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25

Enterobacteriaceae Biochemistry

Background

The **Enterobacteriaceae** are a family of gamma proteobacteria that includes familiar microbial genera like *Escherichia*, *Salmonella*, and *Yersinia*. Members of the Enterobacteriaceae are **bacilli**, and are typically 1-5 μm in length. Like other Proteobacteria they stain **Gram negative** and are **facultative anaerobes**. The ferment sugars (all can ferment glucose) to produce a mixture of solvents, organic acids, and gasses asa end products (varying by strain). Most also reduce nitrate to nitrite via **anaerobic respiration**. Unlike most similar bacteria, Enterobacteriaceae generally lack **cytochrome c oxidase**. Most have many **flagella** used to move about, but a few genera are non-motile. They are **non-spore forming**. Most members of the Enterobacteriaceae produce **catalase**. Some members of this family are able to ferment **lactose** – these are referred to as **coliforms**. Coliforms are less commonly pathogenic, and are used as indicators of water quality. Non-coliforms cannot ferment lactose and are more often act as human pathogens.

We are going to use several biochemical tests to differentiate between (and ultimately, to identify) various members of this family. Here is a little background on the tests that we are using.

MacConkey agar: This is a selective and differential medium. The media contains bile salts and crystal violet dye – both of which inhibit the growth of most Gram positive organisms. It also contains neutral red dye and lactose. Microbes the grow on MacConkey agar may either ferment lactose or not. If they do, the acids they produce turn the indicator dyes red.

If enough acid is produced, the bile salts precipitate and the plate appears cloudy around the areas of growth. If lactose is not fermented, the colonies remain colorless.

Indole: Indole is produced as a breakdown product of the amino acid tryptophan. Indole reacts with Kovac's reagent to turn bright red.

Triple Sugar Iron Agar: This media contains three sugars (0.1% glucose, 1% lactose, and 1% sucrose) along with a acid indicator – phenol red. All Enterobacteriaceae ferment glucose and will turn the entire tube yellow (acid) within 12 hours or so. However, there is very little glucose, so only a little acid is produced. Over the next 12 to 36 hours, bacteria on the slant begin to use amino acids for catabolism. In the process, ammonia (basic) is produced and the pH of the media at the slant becomes neutral (red). However, if lactose and/or sucrose is also fermented so much acid is produced that the tube will remain completely yellow. Gasses produced during fermentation will appear as bubbles or cracks in the media following incubation. Finally, the iron in TSI is used to indicate H_2S production. Hydrogen sulfide produced from breakdown of the amino acid cysteine reacts with the iron to form ferric sulfide (black).

MRVP: The methyl red, Voges Proskauer media is used to detect two different fermentation end products – strains will make one or the other. Methyl red is an acid indicator (it turns red in the presence of organic acids). The Voges Proskauer reagents (A and B) are used to detect acetoin (also known as acetylmethylcarbinol). The test also turns red.

Citrate: This media is used to detect the metabolism of citrate by microbes. In addition to citrate, it contain a pH indicator – bromthymol blue. At acidic pH (citrate present), the media is green. However, if citrate is metabolized, the pH will become basic and the media will turn blue.

Urea: Some bacteria can break down urea to ammonia (basic) and carbon dioxide. This media contains urea and the pH indicator phenol red. Under alkaline conditions, the indicator will turn pink.

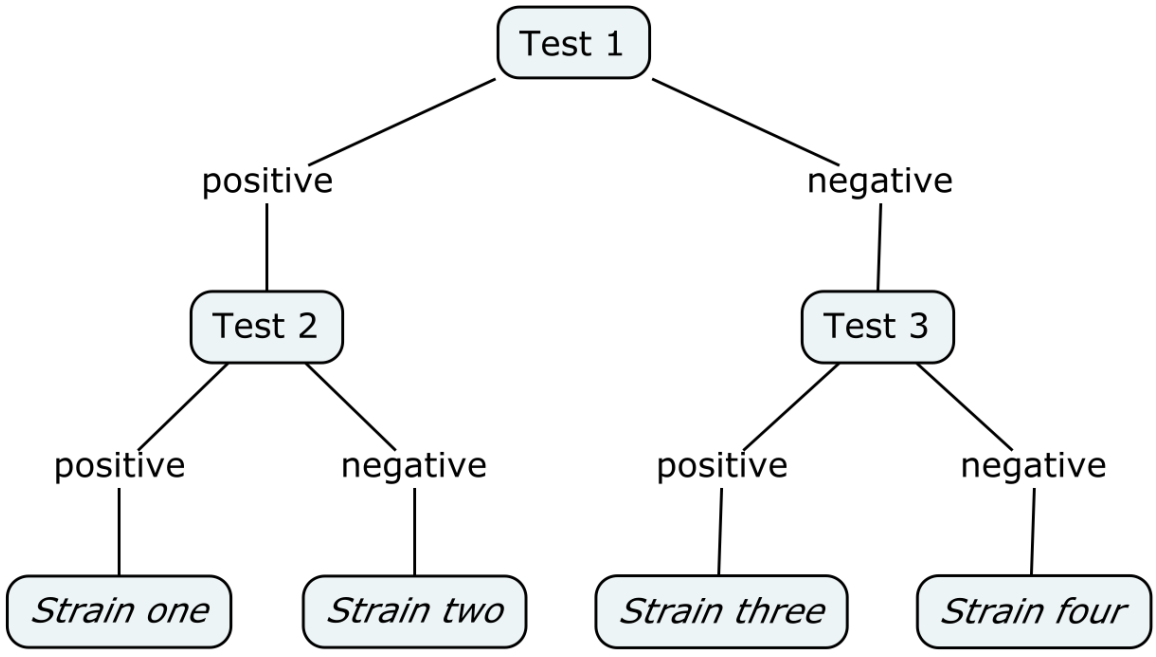
Instructions

Observe each of the sets of inoculated media and interpret the results that you see. The indole test will need to be performed once for each isolate in lab. Based upon the results that you record, you should create a dichotomous key that is sufficient to identify any of the eight bacterial isolates.

For those of you that haven't made or used a dichotomous key in a while – here is a brief overview. The key is actually a decision tree. Each node of the key consists of a question with two (and only two) possible answers. The most efficient keys divide all possible responses into groups of roughly equal sizes. For instance: consider the following.

Test	*Strain one*	*Strain two*	*Strain three*	*Strain four*
Test 1	+	+	-	-
Test 2	+	-	-	-
Test 3	+	+	+	-

The first test differentiates between strains 1 and 2 vs strains 3 and 4. Test 2 can be used to tell strains 1 and 2 apart. Likewise, test 3 and tell strains 3 and 4 apart. Therefore, the dichotomous key could be drawn as show below.



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✪ Observations

• Carefully record the reactions of all the biochemical tests for the eight organisms.

Biochemical Test	Negative result	Positive result	*Citrobacter freundii*	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Salmonella enteritidis*	*Serratia marcescens*	*Shigella sonnei*
Indole	Yellow	Red								
Methyl Red	Yellow	Red								
Voges Proskauer	N.C.	Red								
Citrate	Green	Blue								
MacConkey (Lactose)	N.C.	Red								
Urea	N.C.	Pink								
TSI (Slant)	K - Red	A - Yellow								
TSI (Butt)	K - Red	A - Yellow								
TSI (gas)	N.C.	Cracks								
TSI (H2S)	N.C.	Black								

You might consider including representative pictures of the tests too (either hand drawn or photographs of the media). This could be *very* helpful when you are studying for the laboratory practical exam. Just saying…

✪ Conclusions

1. A complete and correct dichotomous key (based upon your observations) is the only conclusion necessary for this exercise. You will be using this key to identify a bacterial unknown in a future exercise.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Citrobacter freundii* (BSL-1). This microbe is commonly found in soil and water. This species – though not this strain – accounts for many nosocomial infections.
2. *Enterobacter aerogenes* (BSL-1) This organism is part of the normal gut microbiota. It can cause opportunistic infections in immunocompromised individuals.
3. *Escherichia coli* (BSL-1) The K12 strain is the most genetically studied organism on the planet.
4. *Klebsiella pneumoniae* (BSL-2) Strains of this microbe can cause pneumoniae in kids.
5. *Proteus vulgaris* (BSL-2). This microbe is an opportunistic pathogen associated with urinary tract infections.
6. *Salmonella enteritidis* (BSL-2). This microbe is commonly found in bird and reptile feces. It is responsible for most of the salmonella infections that you hear about in the news.
7. *Serratia marcescens* (BSL-1). This microbe is famous for producing a red pigment (Prodigiosin). It is, however, rarely pathogenic
8. *Shigella sonnei* (BSL-2) Strains of this microbe can cause diarrheal infections.

Return the bacterial cultures when you have completed your observations.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

26

Bacterial Unknown Project

Background

This report is meant to be a sort of capstone project for our lab this semester. You will work on this project in pairs. Each person in the pair will be given a mixed culture of bacteria that we have seen this semester. Your goal is to isolate, identify, and describe these microbes.

Instructions

You should keep a record of what you have done, your observations, and your rationale for what you did on each day in your laboratory notebook. You will streak your mixture for isolation (using the BHI plate provided). You will then record their colony morphology (size, shape, margin, color, elevation, and general appearance). You will then select one of the plates to proceed with. Each of you will Gram stain one of your isolated bacteria and record the cell morphology of your isolates (size, shape, arrangement, and stain reaction). You will next perform the appropriate biochemical tests on the bacterium that you stained to identify it. You will also need to create and use a universal dichotomous key for the bacteria that includes your isolate (either Gram-negatives or –positives from before). Finally, you will need to write up a short description of the organisms that you isolated and identified. You should record all of the observations (yours and you partner's) for the mixture that you selected. Don't forget to record the unknown number prominently in your notebook.

Notebook Entry

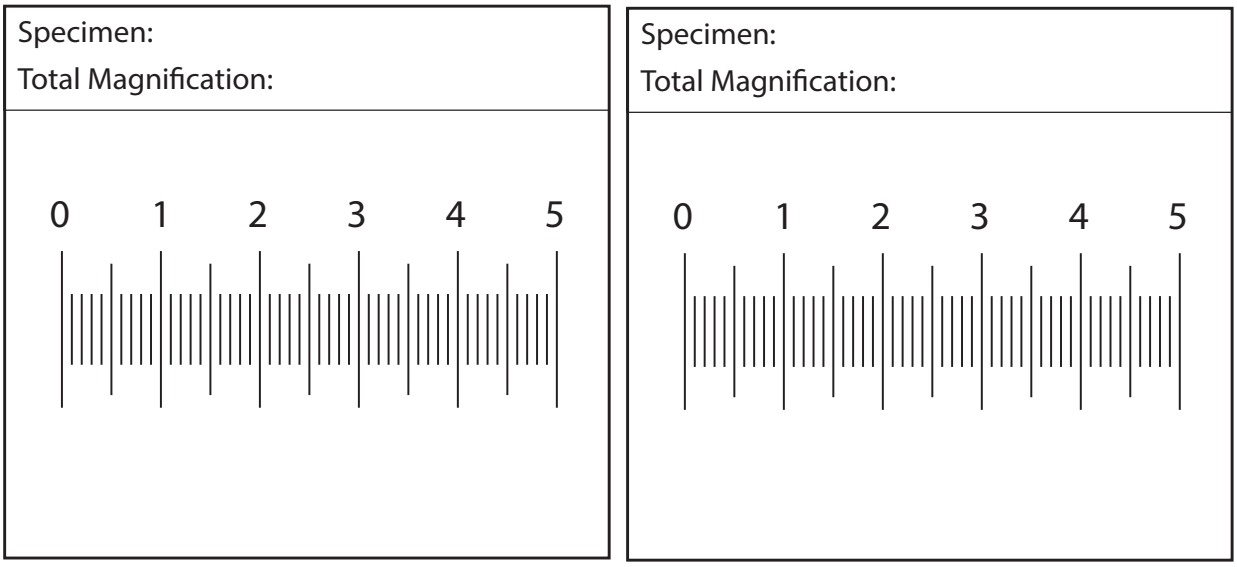
To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-

to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations and Conclusions

Step 1, Streaking: Record (draw or photograph) the appearance of your streak plate. Provide a description of the colony morphologies that you observed. Get one new BHIA plate to subculture a representative of each colony type.

Step 2, Staining: Record (draw or photograph) the appearance of your Gram stain. Use proper terms to describe the cellular size, shape, and arrangement.



Step 3, Characterizing: Record the results of any and all biochemical tests that you performed on the unknowns. You should draw or photograph the biochemical tests to support your interpretations.

Step 4, Identifying: Use your dichotomous keys (from the Enterobacteriaceae and Gram positive cocci exercises) to identify your unknowns. You might consider using a highlighter to illustrate your decision path.

Step 5, Describing: Provide a brief paragraph for each of your isolates to describe what is known about their normal habitat and role in human disease. Remember to use proper scientific nomenclature! That is one of my pet peeves.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Dispose of your microscope slides in the glass waste box when you are finished with your Gram stain. Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. Your unknown contains two of the following:

1. *Citrobacter freundii* (BSL-1). This microbe is commonly found in soil and water. This species – though not this strain – accounts for many nosocomial infections.
2. *Enterobacter aerogenes* (BSL-1) This organism is part of the normal gut microbiota. It can cause opportunistic infections in immunocompromised individuals.
3. *Escherichia coli* (BSL-1) The K12 strain is the most genetically studied organism on the planet.
4. *Klebsiella pneumoniae* (BSL-2) Strains of this microbe can cause pneumoniae in kids.
5. *Proteus vulgaris* (BSL-2). This microbe is an opportunistic pathogen associated with urinary tract infections.
6. *Salmonella enteritidis* (BSL-2). This microbe is commonly found in bird and reptile feces. It is responsible for most of the salmonella infections that you hear about in the news.
7. *Serratia marcescens* (BSL-1). This microbe is famous for producing a red pigment (Prodigiosin). It is, however, rarely pathogenic
8. *Shigella sonnei* (BSL-2) Strains of this microbe can cause diarrheal infections.
9. *Staphylococcus aureus* (BSL-2). This microbe is the leading cause of wound infections. Even our teaching strain is a fairly aggressive opportunistic pathogen. Handle it carefully.
10. *Staphylococcus epidermidis* (BSL-1) This organism is part of the normal skin microbiota. It can cause opportunistic infections in immunocompromised individuals.

11. *Streptococcus pyogenes* (BSL-2) This is the organism that causes strep throat, rheumatic fever, and scarlet fever.
12. *Streptococcus pneumoniae* (BSL-2) This organism is the leading cause of community-acquired bacterial pneumonia.
13. *Streptococcus salivarius* (BSL-1). This microbe is normal member of your oral microbiota. It is rarely pathogenic.
14. *Enterococcus faecalis* (BSL-2). This microbe is commonly found in bird and reptile feces. We used it as a indicator strain (fecal streptococci) in an earlier lab.

Place your unknown in the unlined silver bucket when you have finished all of your streaks.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

Place all used petri dishes into the orange bags in the silver buckets. All glass tubes should be placed in the unlined silver buckets. If you spill a culture, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for

this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

27

Gram Positive Cocci Biochemistry

Background

Like the Enterobacteriaceae, various Gram positive cocci (GPC) can be distinguished and identified using different biochemical tests. We are going to use several different media to differentiate between (and ultimately, to identify) various members of this group. Here is a little background on the tests that we are using.

Mannitol salt agar: This is a selective and differential medium. The media contains 6.5% NaCl – which inhibits the growth of most organisms. It also contains phenol red dye and mannitol. Microbes the grow on Mannitol salt agar may either ferment mannitol or not. If they do, the acids they produce turn the indicator dyes in the media yellow. If mannitol is not fermented, the media remains red.

Catalase: Catalase is an enzyme that decomposes hydrogen peroxide to water and oxygen gas. When hydrogen peroxide is placed on the microbes, the production of bubbles indicates a positive reaction.

EF broth: This media contains a fermentable sugar (glucose) and an acid indicator (bromcresol purple). Glucose fermentation results in acid end products. These, in turn, cause the media to turn yellow. The media also contains a metabolic poison – sodium azide. Only azide-resistant microbes can grow,ferment the glucose, and turn yellow. A negative result remains purple (no growth).

NaCl broth: Like the Mannitol salt agar, this broth contains 6.5% NaCl. Only halotolerant microbes and grow and turn the media cloudy. No growth is negative.

Bile esculin slant: This media is used to detect the metabolism a compound called esculin. If it is converted to esculetin, it will react with iron in the media and turn completely black. A negative result remains tan. Bile acids in this media inhibit the growth of most Gram positive organisms.

DNase: Some bacteria can break down DNA to nucleotides. This reaction is visualized by adding HCl to the DNA plate after microbes have been cultured. The low pH causes intact

DNA to precipitate within the media – turning the plate cloudy. However, any areas where the DNA has been hydrolyzed will not precipitate. This is appear as clear areas around the growth of positive cultures. Negative cultures will not have a clear zone..

Sheep blood agar: Some bacteria can break down blood cells using toxins called **hemolysins**. This results in three patterns of clearing on the plate (**hemolysis**).

Alpha (a) hemolysis: Partial breakdown of red blood cells – resulting in a smoky green color. The plate is obviously not red anymore and it is not clear either.

Beta (b) hemolysis: Complete breakdown of red blood cells – resulting in complete clearing. You can read text through these plates now.

Gamma (g) hemolysis: No clearing. The plates have growth on them, but they are just as red as when they were inoculated.

Two antibiotic disks are also used on our blood agar plate. These are:

A: **Bacitracin** – an inhibitor of peptidoglycan biosynthesis that group A streptococci are particularly susceptible to.

P: **Optochin** – a copper containing compound that pneumococci are particularly susceptible to.

Instructions

Just as before, you should observe each of the sets of inoculated media and interpret the results that you see. The catalase test will need to be performed once for each isolate in lab. Based upon the results that you record, you should create a dichotomous key that is sufficient to identify any of the eight bacterial isolates. See the Enterobacteriaceae exercise for instructions concerning dichotomous keys.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

• Carefully record the reactions of all the biochemical tests for the six organisms. Please record your antibiotic results as either resistant (no zone of inhibition) or susceptible (zone of inhibition). It is much less ambiguous that way.

Biochemical Test	Negative result	Positive result	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Streptococcus pneumoniae*	*Streptococcus salivarius*	*Enterococcus faecalis*
EF broth	Purple	Yellow						
NaCl broth	Clear	Cloudy						
BE slant	Tan	Black						
Hemolysis	Alpha, Beta, Gamma **A, B, G**							
Bacitracin	No zone **R**	Zone **S**						
Optochin	No zone **R**	Zone **S**						
MSA growth	No growth	Growth						
MSA fermentation	Red	Yellow						
Catalase (H_2O_2)	No bubbles	Bubbles						
DNase (HCl)	No clearing	Clearing						

You might consider including representative pictures of the tests too (either hand drawn or photographs of the media). This could be *very* helpful when you are studying for the laboratory practical exam. Just saying…

✪ Conclusions

1. A complete and correct dichotomous key (based upon your observations) is the only conclusion necessary for this exercise. You will be using this key to identify a bacterial unknown in a future exercise.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Staphylococcus aureus* (BSL-2). This microbe is the leading cause of wound infections. Even our teaching strain is a fairly aggressive opportunistic pathogen. Handle it carefully.
2. *Staphylococcus epidermidis* (BSL-1) This organism is part of the normal skin microbiota. It can cause opportunistic infections in immunocompromised individuals.
3. *Streptococcus pyogenes* (BSL-2) This is the organism that causes strep throat, rheumatic fever, and scarlet fever.
4. *Streptococcus pneumoniae* (BSL-2) This organism is the leading cause of community-acquired bacterial pneumonia.
5. *Streptococcus salivarius* (BSL-1). This microbe is normal member of your oral microbiota. It is rarely pathogenic.
6. *Enterococcus faecalis* (BSL-2). This microbe is commonly found in bird and reptile feces. We used it as a indicator strain (fecal streptococci) in an earlier lab.

Return the bacterial cultures when you have completed your observations.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a

paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.

28

Exoenzymes

Background

In catabolism, macromolecules are hydrolyzed to smaller molecules that can then be transported into cells for use as building blocks (as is usually the case with amino acids and nucleotides) or used as an energy source. The enzymes that catalyze these reactions are secreted from bacteria and therefore are considered "exoenzymes", i.e. they function outside the cell. Some important virulence factors of extracellular pathogenic bacteria are also exoenzymes. The following tests examine the production of specific classes of exoenzymes.

Amylase is an enzyme that hydrolyzes **starch (amylose)**, a polymer of glucose, into monomers that can then be transported into cells. After two days of bacterial growth on starch agar, the plate is flooded with **Gram's iodine**, which reacts with the remaining starch in the medium to yield a brown color. If bacteria have degraded the starch in the medium a clear zone will be observed around the bacteria. In some cases, the bacteria may produce or store starch, in which case the bacteria will actually stain darkly.

Interpretation: after adding Gram's iodine

clear zone in medium = + ;

no clear zone = -

DNase activity refers to the ability of bacteria to hydrolyze **DNA** in the growth medium to its component nucleotides. Presumably, these organisms would also secrete a phosphatase to convert the nucleotides to nucleosides for transport into the cells. After two days of bacterial growth on DNA agar, the plate is flooded with **1 M HCl**, which causes the DNA to come out of solution and turn the medium opaque. A clear zone around the colonies indicates that the DNA has been hydrolyzed.

Interpretation: after adding 1 M HCl

clear zone in medium =

no clear zone = -

Gelatinase activity hydrolyzes **gelatin** - a protein produced by partial hydrolysis of collagen extracted from animal tissues. Collagen has an unusual amino acid composition that makes it resistant to many proteases, and thus an organism may secrete proteases that hydrolyze casein, while not exhibiting gelatinase activity. After two days of bacterial growth, the culture is placed into an ice bucket to chill. If the medium does not resolidify (gel), then the organism has hydrolyzed the gelatin.

Interpretation: after chilling on ice

culture remains liquid = +

culture gels = -

Lipase activity refers to the ability of certain bacteria to hydrolyze **triglycerides** to glycerol and fatty acids. Tributyrin (glycerol esterified to three butyric acids) emulsified with Polysorbate (Tween) 80 in a base medium. If lipase is present, the medium around the bacteria will become less opaque. You will be looking for the presence of a halo around the growth.

Interpretation:

Clear zone = +

No change in medium = -

Instructions

Working in groups of four, you will inoculate media to test for each of the four exoenzymes.

Day one: obtain one plate each for amylase, DNase, and lipase.

- Divide the plates into four quarters and label the quarters for the four microbes (e.g. BC, EC, PA, and SA).
- Flame a loop and let it cool. Use the loop to make a simple streak (just one stripe) of each organism in each labeled quarter.
- Obviously, you should re-flame your loop between streaks.
- Incubate the plates (bottoms up) at 35°C.
- Obtain one gelatin tube for each culture.
- Label them with the organism name and your group's symbol.
- Stab them with one loop of culture and incubate them at 35°C.

Day two:

- Treat each of your media as required (see above).
- Determine which microbes produced which exoenzymes.
- Record your results in your lab notebook.

Notebook Entry

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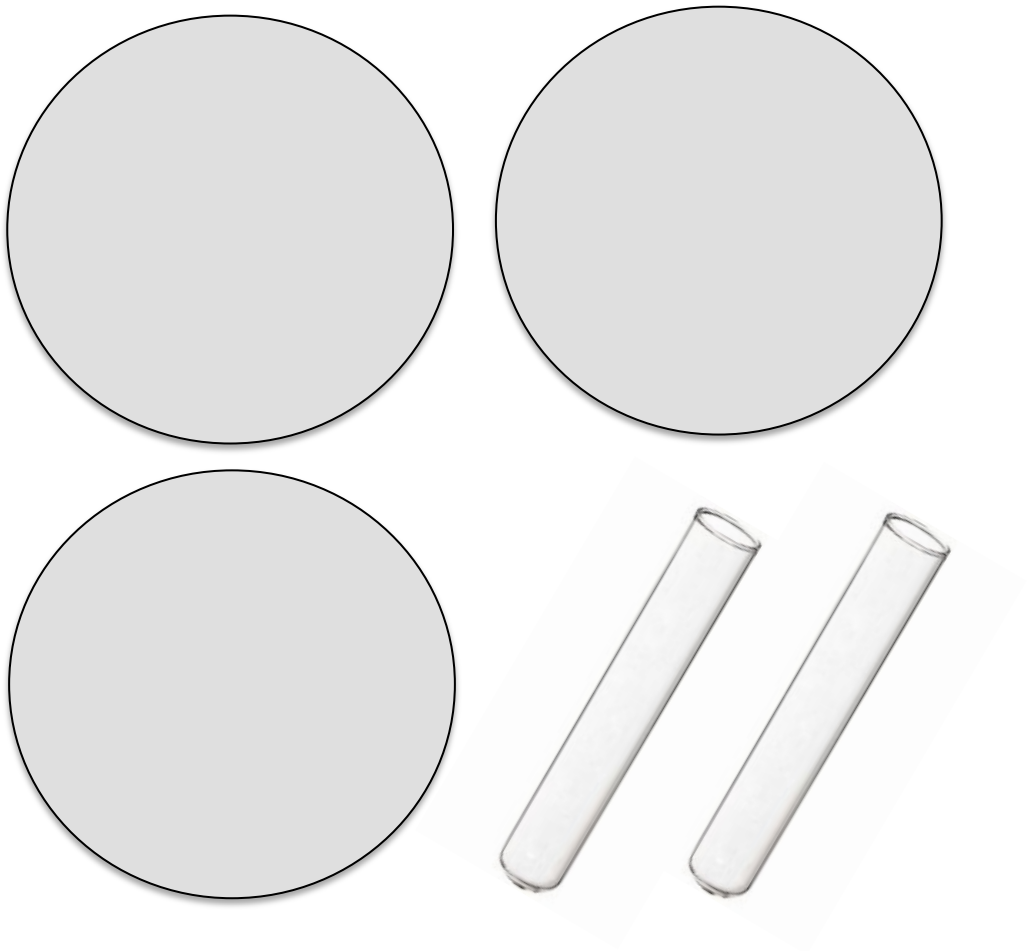
✪ Observations

• Carefully record the appearance of all of your exoenzyme tests. You may record these data in one of two ways:

1) Photograph your media and tape the pictures into your notebook. Make sure that you clearly label all of your media and microbes.



✪ Conclusions

1. Which of these exoenzymes would most likely be involved in virulence in humans? Explain.
2. Which microbes produced which exoenzymes? Provide a table of results.

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Escherichia coli* (BSL-1). The K12 strain is the most studied organism on the planet.
2. *Clostridium sporogenes* (BSL-1) This is basically *Clostridium botulinum* without toxins.
3. *Staphylococcus aureus* (BSL-2) This is commonly found on your skin and in your nose.
4. *Pseudomonas aeruginosa* (BSL-2) This is commonly found in wet environments and can be a potent pathogen under the right circumstances.

Return the bacterial culture when you have completed your stains.

Contaminated materials

You should dispose of your gelatin tubes in the unlined silver cans when you have completed your observations. The petri dishes should all be placed in the orange bap-lined silver buckets when you are done. If you spill any cultures, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

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29

Lysozyme

Background

Lysozyme is a protein found in tears, saliva, and other secretions. It is essentially a peptidoglycan hydrolase and is an important component of our innate immune defenses. This protein is also abundant in egg whites (one of the four most abundant there) and is one of the major contributors to egg allergies.

While most of the body is protected by a covering of dead and keratinized skin cells, the eyes and mucous membranes do not have this protection. Lysozyme is produced in tears and mucus secretions to provide extra protection in these areas against invasion by bacteria. It is also present in the blood to help keep bacteria from being spread throughout the body.

Lysozyme is of historical interest, since it was the one of the first antibacterial substances described by Sir Alexander Fleming. Fleming, who went on to discover penicillin, had a cold and put a drop of mucus on a plate of bacteria. He discovered that the mucus killed the bacteria, but the molecule is too large to be practical as a drug. Many years later, this protein was the first enzyme to have its three-dimensional structure solved.

Instructions

We are going to compare the sensitivity of two microorganisms, *Escherichia coli* and *Micrococcus luteus*, to lysozyme from four different sources. We will use purified lysozyme (commercially prepared), egg whites, tears, and saliva.

You will inoculate your plates with the pure cultures provided; do a 3+1 streaking procedure to ensure a uniform lawn of microbial growth. Divide the plate into quarters on the bottom half using a sharpie. Label each quarter for one of the four sources, and add your initials and lab section number to identify your plate later. Use the onions provided in lab to induce tear formation and touch a sterile paper disk to one tear (not directly to your eye – ouch!). Place that disk in the tear sector of your plate. Touch the other disks to the

other sources and place them on the corresponding quarters of your plates. Incubate your plates at 35°C, bottoms up, until next time.

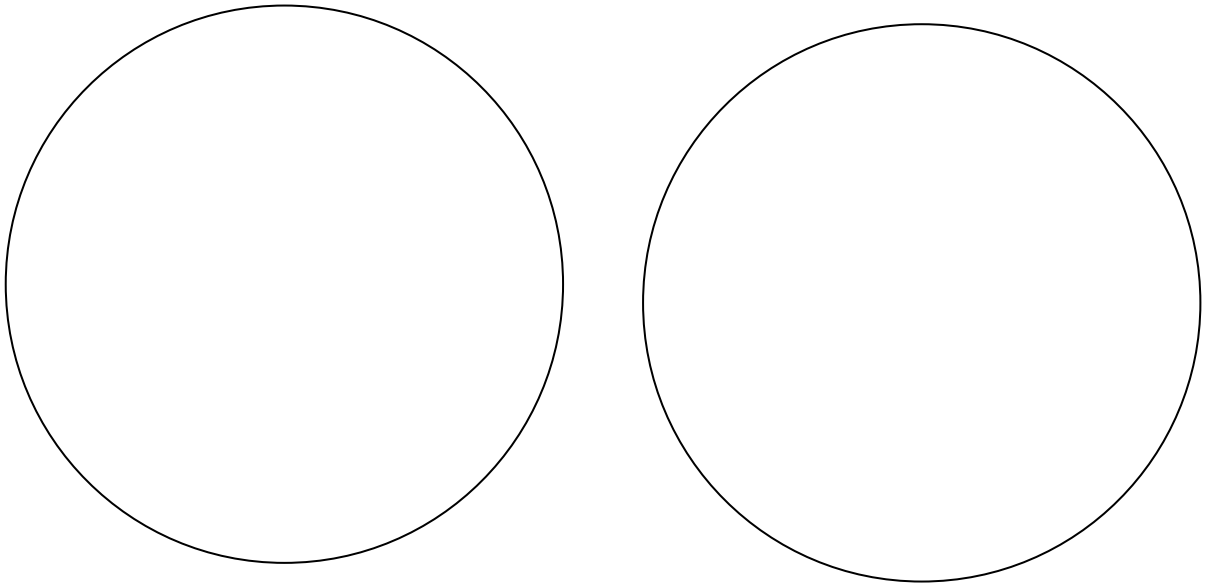
Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

✪ Observations

• Carefully record the appearance of your *Micrococcus* and *Escherichia* plates. You may record these data in one of two ways:

1) Photograph your plates and tape a copy in your notebook. Make sure that you clearly label your samples.
2) Using the panels below, draw an accurate representation of each sample. Make sure that you label each sample well.



✪ Conclusions

1. Which microorganism was more resistant to lysozyme?
2. What is the most likely reason for this result?
3. Which source of lysozyme gave the best results?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Any broken glass should also be place in the glass waste box. **Do not** put anything sharp into the regular trash.

Bacterial cultures

We will be working with live microorganisms in this exercise. If spill the culture or otherwise contaminate yourself, you should disinfect your bench with Roccal and wash the contaminated area with plenty of soap and warm water. The cultures we are using include:

1. *Escherichia coli* (BSL-1). The K12 strain that we use in our laboratory is not especially virulent. It is the most genetically studied microbe on the planet.
2. *Micrococcus luteus* (BSL-1) This bacterium is a gram positive saprophyte and is a member of the normal microbiota of human skin.

Return the bacterial cultures when you have completed your inoculations.

Contaminated materials

After making your observations, you should dispose of your petri dishes in the silver cans that are lined with the orange biohazard bags. If you spill any of the cultures, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

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Hand washing

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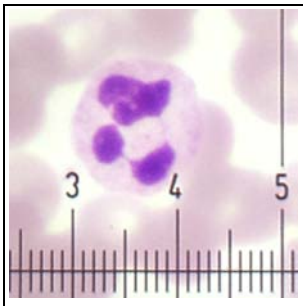
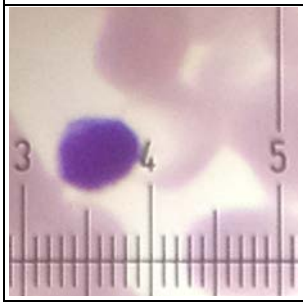
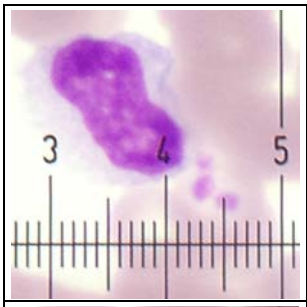
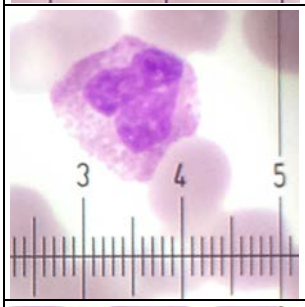
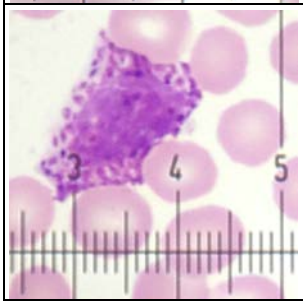
30

White Blood Cells

Background

Many cells of our immune system are associated with lymphatic tissues (lymph nodes, the spleen, etc.). Other important immune cells are found in our circulation. These are the leukocytes, or "white blood cells". There are five different types of white blood cells in human blood, each performs a different function and is present at different levels. A normal person has between 5×10^6 and 1×10^7 white blood cells per ml. In addition to being important for immunity (obviously), imbalances in leukocyte levels can provide clues to underlying disease conditions. In today's exercise, you will determine the relative abundance of leukocytes in a prepared slide of human blood. This is referred to as a differential blood count. The five types of cells that you will be counting are described in the table below.

	**Neutrophil** – (approximately 62%) This is the most common white blood cell. Neutrophils are a type of granulocyte and are the main phagocytic cells in our circulation. You can recognize these by their lobed (3-5 lobes connected by thin strands of nuclein) nucleus. Elevated neutrophil numbers (neutrophilia) are usually indicative of a microbial infection.
	**Lymphocyte** – (approximately 30%) These include the B-cells and T-cells of the adaptive immune response. Microscopically, you cannot differentiate between the types of lymphocytes. Elevated numbers, lymphocytosis, can indicate an infection (or more rarely – cancer).

	**Monocyte** – (approximately 5%) These immune cells ultimately differentiate into tissue macrophage and dendritic cells. They are important antigen presenting cells. Elevated levels, monocytosis, can be caused by chronic infections (such as syphilis or tuberculosis).
	**Eosinophil** – (approximately 3%) These cells are filled with pink stained granules. Their function is primarily to deal with worm infections (like trichonosis). Unfortunately, they are also associated with type I hypersensitivity (allergies). Either of these conditions will lead to elevated levels (eosinophilia).
	**Basophile** – (<1%) This is the rarest of the circulating leukocytes. These cells are filled with granules that stain purple (and often obscure the view of the nucleus). Elevated levels (basophilia), is associated with anemia and some microbial infections (like chicken pox).

Instructions

Observe one of the commercially prepared human blood slides using your high dry (40X) objective. You may use oil if you wish (the photo that I took – see above – were made using the oil objective).

- You will need to find one example of each type of blood cell and record its appearance.
- Count between 100 and 200 leukocytes and tally the number of each type.

Notebook Entry

To receive full credit for your work, your entry must contain the eight required components discussed in "Keeping a Notebook". As a reminder, these sections are: a Table of Contents entry, title, the purpose, a procedure, your observations, your conclusions, the date, and the signatures. Take a moment to ensure that your notebook is complete and up-

to-date. The most critical components are your observations and conclusions. Specific guidelines regarding your entry for this exercise are given below.

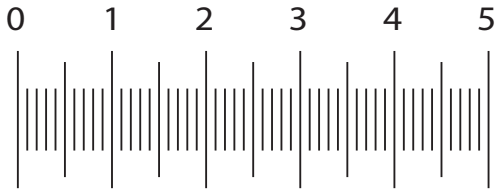
✪ Observations

• Carefully record the size, shape, and appearance of each of the white blood cells in your slide. Search around your slides and make sure that you all five types – you might have to hunt a while to find a basophile.

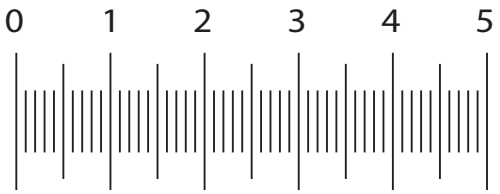
You may record these data in one of two ways:

1) Using the panels below, draw an accurate representation of each cell. Make sure that you label each one well. Also ensure that your drawings show the correct size, shape, and color of each cell type.

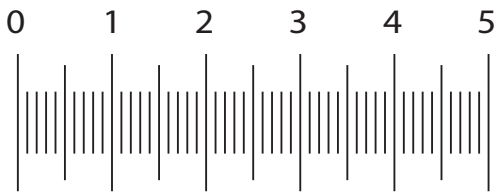
Specimen:
Total Magnification:



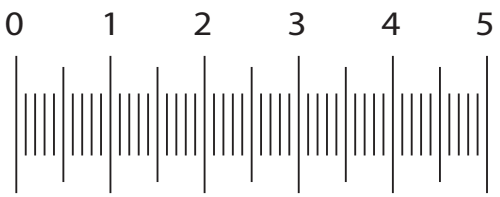
Specimen:
Total Magnification:



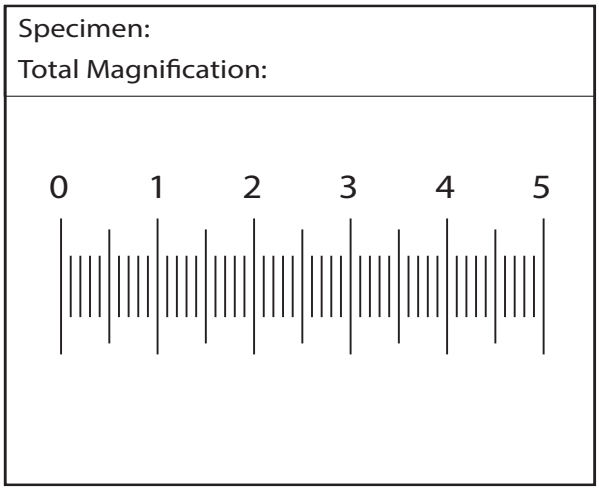
Specimen:
Total Magnification:



Specimen:
Total Magnification:



2) Using your cell phone, take a picture of each cell type. Ensure that you also capture the ocular micrometer to calculate the cell's size. Print your photos and tape them into your lab notebook. Make sure that you label each of your photos.



You should also count between 100 and 200 white blood cells as you slowly and methodically scan your slide. Record the number of each type of cell that your observe in a table like this one.

Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil

✪ Conclusions

1. Were the percentages of each cell type similar to the normal range?
2. What would happen to the cell distribution if this individual had severe allegies?
3. Which of these cells is/are most associated with the adaptive immune response?
4. Which of these cells is/are phagocytes?

Housekeeping

To keep our laboratory clean, safe, and organized, we ask that you adhere to some basic housekeeping protocols. Please ensure that you complete each of the following when you have finished working on this exercise for the day.

Glass waste

Any broken glass should be placed in the glass waste box. **Do not** put anything sharp into the regular trash.

Commercial Slides

Do not throw the commercial slides or the hanging drop slides away! Wipe any immersion oil off of the slides using a Kimwipe. Then return the slide to the correct slide box. Return the hanging drop slides to the indicated container for later cleaning.

Compound light microscope

Before returning your microscope to its respective bin, you should do the following:

1. Unplug the power cord and wrap it around the brackets on the microscope's arm.
2. Rotate the head of the microscope so that the ocular lenses face toward the arm.
3. Clean any immersion oil off of the 100X objective lens using lens paper.
4. Rotate the objective turret so that the 4X (red striped, scan) objective is facing down.
5. Using the course focus knob, lower the microscope stage as far as it can go.
6. Carefully return your microscope to the correct cubby. Please orient the microscope such that the arm of the instrument faces out.

Contaminated materials

We should not contaminate anything today. If you do, however, soak the area with Roccal. Then clean up with paper towels and dispose of the mess in the lined silver buckets.

Laboratory bench

You should disinfect your lab bench at the beginning and end of each class session. Squirt a small amount (a few milliliters) of Roccal onto your bench top and wipe the surface with a paper towel. Dispose of the paper towel in our regular trash can. If you spill a microbial culture on the bench, spray the area with Roccal and allow to sit for two minutes. Then wipe the area with a paper towel and dispose of it in the silver bucket lined with the orange biohazard bag. Start doing this today.

Cell phone / camera

Before leaving the lab for the day, you should take a moment to disinfect the cellphone or camera that you used to make observations. Antimicrobial wipes are available in the lab for this purpose. Wipe all sides of your device with a single wipe and then dispose of the wipe in our regular trash can. Start doing this today too.

Hand washing

You should always wash your hands with warm water and soap for 30 seconds before leaving the lab for the day. Dispose of the paper towel that you dried you hands with in the regular trash can. You should do this today (and ***every day***) in the lab.