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| **Tabletop Lab:** | **Microbe Diversity** |

You will need one additional resource, which can be found in the course module:

* **Microbe Videos:** This is a collection of videos that will help you explore the world of microbes without the benefit of a microscope.

You will need the following supplies that you should have purchased or obtained through the college:

* 4 petri dishes with tryptic soy agar

Collect the following supplies from home:

* 5 large objects, such as bricks, blocks of wood or cans
* Index cards and large marker

You will be conducting an experiment in this lab that requires 48 hours incubation, so be sure to read through all the instructions before starting any of the lab activities or attempting the self check quiz associated with this assignment.

**Introduction**

Early in the history of life, single-celled organisms diverged into two groups, the Bacteria and the Archaea. Both kinds of organisms are characterized by small, simple prokaryotic cells. But they are so different in other ways that they are divided into two separate domains; ***Domain Archaea*** and ***Domain Bacteria.*** Organisms with larger, more complex cells, characteristic of plants, animals and protists are placed in ***Domain Eukarya***. Archaeans, the least familiar, appear to be most similar to the earliest living cells and are found primarily in extreme environments, such as hot springs.

Species in the Domain Bacteria consist of ***prokaryotic*** cells, meaning those without a nucleus or other membrane bound organelles in their cell structure. They are considered less primitive and are relatively well known by microbiologists, because they are typically easy to grow/culture in the lab. Bacteria are classified in many ways; one of the most common is gram staining. This procedure divides all bacteria into gram-positive and gram-negative groups based upon the absorption of dyes by the cell wall. Another distinction is the shape of the cell; usually spheres (**cocci**), rods (**bacilli**), spirals (**spirilla**), or curved (**vibrios**). Familiarize yourself with the basic shape patterns for bacteria using your textbook and lecture material.

In an online lab, you do not have access to a microscope. Instead, you will go through a variety of activities to better understanding the basics of microscopy, microbial diversity, and comparative cell size. To obtain the best view of bacteria under the microscope they must be "fixed" (killed and stuck to a glass slide) and usually stained with special dyes to make them more easily visible. However, it is possible to view bacteria while living. To see examples of various microscopic life forms, you should view the Microbe Videos found on the course website, in the Microbe Diversity module before starting the lab activities.



**Part 1: Introduction to Microscopy**

Although nothing can replace the hands on experience of working with a real microscope, there are many virtual microscopes online that can be used as a learning tool. One such virtual microscope was developed by the Univeristy of Delaware, and it is one of the more robust interactive simulations out there. To become familiar with the parts and operation of a microscope go to the following website:

Your task is to complete the tour of the microscope, while learning how to load and view microscopes slides, adjust the focus, and find specimens in the viewing field.

After gaining practice working the microscope with the guided tour, you will apply what you learned by attempting to find specimens in several challenge slides.

<http://www.udel.edu/biology/ketcham/microscope/scope.html>

**Procedure:**

1. Complete the guided tour by following along with the tour guide as he explains all the steps in the “Getting Started” inset to the right of the microscope. You can turn on Closed Captioning by clicking on the CC button in the inset. Hint: When you are instructed to “Click here to…” this means you should click on the item in the Getting Started inset that is outlined in red.
2. Once all items in your checklist are completed, you are ready to try working the scope on your own. Once you have exited the Getting Started tutorial, you will see 4 microscope slides in the top right hand side of the screen. Practice your microscope technique by viewing each of these slides. Each slide has a different type of cell. While exploring these slides make note of some of the qualitative characteristics: Shape, relative size, visible structures, etc.
3. Now click on the “try this” icon on the left hand side of the page. There are 6 puzzles for you to try. Click on each puzzle in turn to find the specimen in the scope, using what you learned in the tutorial. Hint: Don’t forget that you can switch views to see whether your specimen in centered over the light aperture. For each specimen, write a brief description below the steps required to focus in on your specimen.
   1. p1:
   2. p2:
   3. p3:
   4. p4:
   5. p5:
   6. p6:
4. In addition to the 6 puzzles, there 3 slides that you will attempt to take measurements on. Select each slide in turn and take the measurements outline in the simulation. Record the correct size of each object you are instructed to measure below.
   1. Height of the letter e:
   2. Width of the onion cell:
   3. Diameter of the air bubble:
5. The slide that you measured the air bubble, there was images of bacteria. Based on the measurements you took, how does a bacteria size up next to an onion cell?

**Part 2: Relative Size of Microbes**

Your goal in this exercise is to develop an appreciation and understanding of the comparative size of microbial life.

For this activity you will need to find a large area such as a long stretch of sidewalk near your home, a parking lot or a gym floor. Many different places lend themselves to this activity, but ideally it should be a place that does not have a tremendous amount of foot traffic.

You will need:

* Five objects that will be used as a marking devices that can be seen at a considerable distance such as bricks, canned food, a block of wood, or whatever you have around the house.
* Paper and pencils to make signs (index cards and a wide magic marker would be ideal.

Most bacteria are measured in a unit called micrometers. This unit of measure is 0.001 of a millimeter (the smallest measurement on a normal meter stick). Another way to think of a micrometer is that it is one thousandth of a millimeter, a very small space indeed and one that cannot be measured with any kind of measuring stick – yet for proper classification of microorganisms a microbiologist must be able to communicate information about their sizes.

This is done on very sophisticated optics with a device called a ***micrometer***. This is a measuring tool that divides the viewing area of a microscopic field of view into equal and precise small distances on high magnification levels. For this exercise though we will not be using a microscope, which can be very expensive and requires in-person training to learn to use well. Instead we will simulate these microscopic distances with our feet.

**Procedure:**

Generally speaking a normal adult’s stride (1 large step) is approximately 1 yard (just shy of a meter). You may want to be more precise and if you have a measuring tape such as those that are used in construction or sewing handy then take an average distance of 10 of your strides in succession and estimate your exact stride distance (this is not required to learn about the size of microbes in this exercise however).

1. Place a marker (can or brick) as your starting place.
2. Count out 100 strides and place another marker.
3. With your sign material make a simple label called “Human Hair width.” This distance will represent the distance that an average human hair would be when considering the distance across the hair shaft (not the length).
4. Return to the start and count out 10 paces. Place another marker.
5. Make a label called “Human red blood cell.”
6. Return to start and count out 2 paces. Place another marker.
7. Make a label called “ E. coli” – this is a bacteria found in the digestive system of many animals yet it can also cause diseases that may be deadly. Escherichia coli can be deadly to humans if they are misplaced (such as contaminated food or water) yet they are also necessary symbiotic bacteria in the digestive system of cows to help them digest all the roughage in their diets. E. coli are considered giants in the bacteria realm and often are used to help beginning students learn to identify bacteria when using microscopes.
8. Return to the start and count out a half pace (about 0.5 m). Place a marker.
9. Label this site as Staphylococccus. This is a common bacterium in the natural environment that can cause food poisoning and is what people are referring to when they speak of a “staph infection.” In highly antiseptic environments, such as hospitals, staphylococcus can become antibiotic resistant and lead to a host of problems that are hard to cure; sometimes staph infections will even invade the skin causing painful sores. A good one to know about and best avoid if possible.
10. Finally the last measurement involves removing your shoes (or at least one).
11. Return to start and place your naked foot behind the starting marker.
12. Allow just your big toe to just cross the starting line. Place your last marker here (this will be approximately 2 cm or just under one inch). There are 2.54 cm in an inch, so unless you have a really large big toe, the distance of your toe nail covering the toe should be about 1 inch long.
13. Make a label called virus. Most viruses can vary tremendously from 0.02 micrometers to 0.20 micrometers or 1-10 inches in this comparable distance exercise. The common cold virus is around 0.09 micrometers or about 4 inches long in this exercise.

**Analysis:**

Consider the comparable length of the human hair, the human red blood cell, two types of bacteria and a representative virus.

1. Approximately how many times larger is a red blood cell compared to a large bacteria cell of E. coli?
2. Approximately how many times smaller is a staphylococcus bacteria compared to a red blood cell?
3. How many staphylococcus bacteria could stand in a line across the width of an average human hair? Does this number surprise you?
4. What is the smallest distance that a traditional light microscope can detect? Hint: you may need to consult the internet.
5. In your line, where would you need to draw the line for what can be seen with light microscope based on the answer to the question above? I.e. Which of your labels, or organisms, can you view in a light microscope, and which organisms can you not view in a light microscope? What type of microscopes detects organisms below this threshold?
6. If one human pace of about 1 meter equals 1 micrometer or 0.001 m, how many paces would be necessary for the following organisms to be represented in our footpath model?
   1. Paramecium (a common food protozoan in pond food chains) is 200 micrometers long.
   2. HIV virus is \_\_\_\_\_\_\_\_\_ long.
   3. Bacteriophage (a virus that infects bacteria and is used for transferring genes in biotechnology) is 0.06 micrometers long.

**Part 3: Culturing Microbes**

You will need to obtain 4 petri dishes with tryptic soy agar (which is a shallow, plastic dish with jello-like material that is sterile). You will use this to test microbes that may be growing on your skin and around your home. To prevent contamination, do not open this petri dish until you are ready to transfer the suspected swabs of microbes into it. (If your petri dish has mold or other visible growth, contact your instructor early in the week to get a replacement, because your petri dish is no longer sterile and you should not open it). For this activity you will need 2 clean, Q-tip swabs to transfer the bacteria to the plate, have these ready before you begin. You will also need a very small quantity of clean water.

**Procedure**

1. With a marker, draw a line down the middle of one of the plates (on the outside) on the lower side that contains the gel. This line is going to divide your samples into 2 halves. On one half, label it "normal" and the other half label "clean."
2. Take the first swab and moisten it with clean water, bottled water is ideal. Rub this swab over your hands, including under the fingernails If possible, along the cuticles and anywhere that touches everyday normal surfaces.
3. Remove the lid of the petri dish, and gently rub the swab from your "normal" (unwashed) hands onto the gel, without breaking or tearing the gel, making a wavy like pattern on the "normal side."
4. Next wash your hands thoroughly, using soap and warm water to clean your hands. This will considered the "treatment" in our experiment, because you will treat your hands with sterilizing materials to see if they eliminate germs.
5. Repeat step 2 with your now very clean hands. Gently inoculate the second half of the petri dish with a wavy pattern with this sample.
6. Reseal the petri dish with tape, and place into your warm incubating area (ideally on top of the refrigerator at home) and culture for 48 hours. Do NOT open the petri dish for any reason to see the bacteria or fungal growth up close, this can potentially be hazardous.
7. With the second petri dish in the kit, swab a place in your home that you would like to view bacterial growth. Behind the kitchen sink, a window sill, the floor near where animals are fed etc, are all great places to swab for a culture. Again though it is critical to NOT open the petri dishes after you have cultured them in order to prevent contamination.
8. Clean all surfaces in your home that may have come into contact with the microbial set up with a disinfectant to avoid any possibility of contamination in the home.
9. With the extra dishes, you can recruit a volunteer to test a different type of hand cleanser. Set up the second plate just as you did in step 1. If you used regular hand soap in your first plate, use hand soap that is labeled as “Anti-Microbial” or vice versa. This type of cleanser has a chemical additive, typically called Triclosan. (Check the active ingredients on the label to verify it contains anti-microbial chemicals.)
10. After 48 hours, view the results and write a brief and detailed statement on your observations in your lab notes. Make note of color, distribution, unusual features and differences in the two halves of each of your plates. If there are not noticeable differences, hypothesize on why, i.e. poor hand washing technique, dirty hand towel (this is the most common source of contamination in this experiment when done at home), etc. Be sure to indicate where in your home you obtained your second swab sample or if your tested an anti-microbial product. Again, do NOT open the petri dishes while you make your observations to prevent exposure to unwanted contamination.
11. To dispose of the petri dishes at the conclusion of this lab, place all of them into a Ziploc type baggie, seal and then dispose of in the trash. Do not allow children or others to open and view or play with the microbial materials, in case there is any possibility of contamination with infectious materials from the home environment. Dispose of right away after you make your initial observations.

**Part 4: Protista**

**Dichotomous Key of Protists**

Although also singled-celled, protists (Domain Eukarya, Kingdom Protista) are eukaryotic. Their cells are significantly more complex than the prokaryotic bacteria and archaeans. Most are free living but others are parasitic and are responsible for some of the most serious and deadliest of human diseases. Protists are most often found in association with moist environments, which range from oceans and ponds to the human bloodstream. In today's lab we will examine pond water and lab cultures to gain a better understanding of the members of this important kingdom.

**Procedure**

1. View the protist video in the Microbe Diversity module of the course website.
2. Use the protist key at the right hand side of the page to identify at least four of the organisms featured in the video clip. Fill out the table below. In the first column, give a description (in your own words) of each organism you choose, making note of shape, color, motility, and any identifying or unusual features.
3. Follow the dichotomous key to identify your chosen organisms. In your notes, describe the sequence of steps for how you would identify the specimen (seen in lieu of not having a microscope).
4. Sketch two different species seen in the video. Creating a detailed sketch will help you gain keen observation skills.

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| **1a** Organism attached by stalk  **1b** Organism without stalk; free-swimming, grouped , floating, or as a filament | Go to 2  Go to 3 |
| **2a** Stalk supports large, colonial ball or cattail-like structure  **2b** Stalks support small cell having either tentacles or cilia as viewed with a microscope | **Slime Molds**  **Ciliates** |
| **3a** White or colorless  **3b** Colored | Go to 7  Go to 4 |
| **4a** Cells are green and contain chloroplasts  **4b** Yellow, orange, or white gelatinous or netlike mass | Go to 5  **Slime Molds** |
| **5a** Overall threadlike or treelike appearance; cells arranged either end-to-end as a filament or with branching filaments  **5b** Cells not arranged in filaments | **Chlorophyta**  Go to 6 |
| **6a** Single cell or group of cells, with yellow pigments and distinct glass-like walls with grooves or holes  **6b** Single cell or group of cells, either motile or motionless and lacking glasslike walls | **Chromista**  **Chlorophyta** |
| **7a** Exhibits definite motion  **7b** No motion, spherical shell with spines | Go to 8  **Ciliates** |
| **8a** Slow-creeping  **8b** Exhibits some other motion | **Ameoba**  Go to 9 |
| **9a** Cells propelled by one or more flagella  **9b** Cells with many cilia | **Euglenoids**  **Ciliates** |