

Getting Ready to Do Microscopy

Lesson Title: *The Ecology of Your Skin 3: The Body Food Connection*

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Teacher Sheet

The plan is to use compound microscopes, the best ones available to you. A 40X objective lens is the minimum needed. If, in addition, you have a 100X oil immersion lens, that's better. It can be quite a challenge to teach students how to find bacteria under the microscope. If you have not done it in awhile yourself, try it out following these rules of thumb (this is pretty much the set of instructions for students, too, except they include some of the trouble shooting instructions that you will need to guide the students through it):

1. Use a toothpick to get a sample from between your own teeth.
2. Dab the sample onto a slide with a tiny drop of water. (Don't let the students make a big watery preparation!)
3. Add a coverslip and let the preparation sit for 3-5 minutes to give the bacteria a chance to settle down on the glass. They will be easier to see and this is where they prefer to be.
4. Start with the lowest power (e.g., 4X) objective and get a tiny particle of debris into focus. This is essential no matter what sample you have. If you cannot see it under 4X, you will not see it at higher powers. Start with lowest power and get that right.
5. Move to your next power (e.g., 20X) and notice that you may need to adjust the light. The bacteria are transparent and too much light will shine right through them. Bacteria may not be visible yet, especially to untrained eyes. Focus on a bit of debris.
6. At 40X (adjusting the light a little more), begin to search for bacteria. They will be an order of magnitude smaller than microbes you might typically look at, such as Paramecium. Relax your eyes (your focus) and let yourself go down to the level of tiniest, palest objects. If they are rod shaped, moving or not, you are there! Stay there and look for little spheres and spirals and even elongated, thread-like rods and elongated helices of spirochetes.
7. If you have an oil immersion 100X lens, the next step is a bit more involved. It is essential that the preparation not be too watery and that you have given the bacteria a chance to settle to the glass surface.
 - a. Move the objective lenses aside.
 - b. Apply a tiny drop of microscope immersion oil to the coverslip over the area you plan to view.
 - c. Click the 100X into place and, looking from the side, slowly lower that lens into the tiny drop of oil just to the point where the lens and the oil touch.
 - d. Now look through the eyepiece and fine-focus gently and slowly, looking for bacteria, and maybe adjusting the light.
 - e. When you are done with the 100X objective, gently wipe off the oil with lens tissue (not kimwipes or facial tissue because they are too rough).

Where to Collect Body Bacteria for Direct Viewing under the Microscope

1. Between the teeth is the first choice because it is a wet area teeming with bacteria and therefore increases the likelihood that the exercise will be a success.
2. Other parts of the body are good choices only if they are quite damp! That will assure that you have enough bacteria in a tiny sample that searching under the microscope will be rewarding. Ordinary hygiene practices dictate that various accessible parts of our bodies not be especially damp! If you'd like to assign the students to NOT put on their

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underarm deodorant in the morning, but rather to carry it with them to school and apply it right after your class, then possibly you can have them sample a sweaty armpit with a cotton swab. However, even this is not ideal as there is likely to be a residue of antibacterial activity from repeated, prolonged applications of underarm products. Abstaining one day may not be enough.

3. A very rewarding sample, teeming with large, active bacteria may be prepared by leaving some chicken or beef broth in an open container for about three days. Although the bacteria you will see are not directly relevant to the ecology of the skin, they are a good starting sample to guarantee that your students will see something.
4. If you have access to supplies and equipment for making nutrient agar, you may use that as a way to grow large numbers of bacteria isolated from various body parts. Those large numbers will be readily visible under the microscope.
 - a. Dip a cotton swab into sterile, distilled water or (if that isn't available) boiled (then cooled) water. (Note a cotton swab right from the package is sterile enough.)
 - b. Wipe the moist swab across the body surface of your choice.
 - c. Wipe the swab across the surface of a plate of nutrient agar.
 - d. Allow the plate to incubate either at room temperature or at 35 degrees centigrade (around body temperature).
 - e. In a couple of days, white and yellow colonies will be visible. These may be picked up with a toothpick (straight from the package is sterile enough) and placed on a slide in a tiny drop of water and topped with a coverslip for viewing.
 - f. Note: discarding the Petri dishes and used toothpick may be safely done by pouring on a little bleach and then wrapping the plates in a plastic bag and discarding. Alternatively, if you have access to an autoclave, use that to sterilize the plates before discarding.