15: Hanging Drop Wet Mount

Objectives

- Learn how to perform the motility test.
- Differentiate between Brownian movement and true motility.

Motile bacteria move about with structures called **flagella** (a few exceptional bacteria move with the help of **axial filaments**, which cannot be seen in the microscope). Nonmotile bacteria without flagella are called **atrichous**.

Motility can be identified in a couple of different ways:

- the hanging drop wet mount?
- motility agar media (SIM and tetrazolium motility agars covers in Motility agar exercise)

Looking at living bacteria are not as easy as one would think. First of all, living bacteria have no color, and they are small: therefore, they are really difficult to see, even with the oil immersion lens. Second, all bacteria have some vibrational movement, even nonmotile ones. This **Brownian movement** is caused by water molecules bouncing around in the solution, knocking up against each other and the microorganisms. Kinetic energy inherent to all molecules causes this kind of movement. On the other hand, those bacteria with flagella will be very apparently moving about the field of vision, although perhaps not all of the bacteria will be moving. Some cells will "run" straight across the field, others will "tumble" across the field in a slower motion.

The keys to a good hanging drop slide are 1) use a small drop of bacterial suspension, but do not let it dry out, and 2) use a young culture of bacteria.
MATERIALS NEEDED

- fresh cultures (broth medium less than 24 hours old is optimal) of various bacteria (assigned by instructor)
- depression slides
- cover slips
- petroleum jelly

THE PROCEDURE

1. Place a drop of the bacterial culture (optimally from a young broth culture) in the middle of a cover slip.
2. Place a thin line of petroleum jelly around the edge of the cover slide, or at the 4 corners of cover slip.
3. Turn the depression slide upside-down (depressed area facing down) and gently touch the cover slide. The jelly holds the cover slip to the slide and also keeps the suspension from drying out.
4. Now flip the entire microscope slide/cover slip combination over. It should look like the diagram below.

QUESTIONS

1. Why is petroleum jelly used to make this slide?
2. What is the cause of Brownian movement?
3. What feature of the bacterial culture will increase the probability of true motility?

Contributors

- Jackie Reynolds, Professor of Biology (Richland College)