Microbiology Lab Experiment Changes

Experiment #: 3-10, 3-11, 5-22

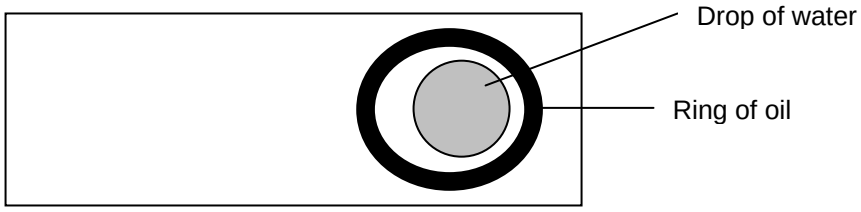
Title: Motility: Hanging drop & Motility Agar Deeps

Live Organisms: *Escherichia coli; Micrococcus luteus; Aquaspirillum itersonii* (broth); *Corynebacterium xerosis; Staphylococcus aureus*

Part 1:

Changes:

1. Instead of a hanging drop slide we will suspend the bacteria in a circle of immersion oil.



2. Carefully suspend the bacteria in the drop of water by touching the loop to the drop. Try to avoid mixing otherwise you will mix the oil and water together. Your suspension should be **slightly cloudy!** (Note: If using a broth culture, don't put a drop of water on the slide. Use several loopfuls of the broth culture instead.)

3. Apply a coverslip by "plopping" it on top. You want to trap air bubbles!

4. Focus on the edge of an air bubble. Close the iris. Go up to only 400x. Using oil is not necessary. On one side of the bubble will be air. On the other side will be the bacteria.

Part 2:

Another technique to determine motility involves observing the movement of bacteria thru motility agar.

1. Motility deeps have tetrazolium chloride (TTC) added to the agar. This helps us to visualize the bacteria in the agar.

2. Using the same organism as you used for the wet mount, carefully stab a motility agar deep. Your instructor will demonstrate. The tubes will be incubated at least 48 hours.

Take Home Lesson: The presence of bacteria in the agar is indicated by a red color. The red color is the result of bacterial metabolism. TTC accepts electrons from bacteria. When oxidized, TTC is colorless and soluble. When reduced, TTC is red and forms an insoluble precipitate (i.e., it does not diffuse thru the agar). This means that wherever you see red the bacteria are there. The red color is not intrinsic to the bacteria. It is caused by the reduction of the chemical tetrazolium chloride as a result of bacterial metabolism.

In a microscope, distinguish true bacterial motility from random Brownian movement. Did your motility agar match your wet mount results?