

Examination of Living Microorganisms

EXERCISE

2



MICROBOME

OBJECTIVES

After completing this exercise, you should be able to:

1. Prepare and observe wet-mount slides and hanging-drop slides.
2. Distinguish motility from Brownian movement.

BACKGROUND



ADAPT: Demonstrate ability in properly preparing slides for microbiological examination including performing wet mount and/or hanging drop preparations and recording microscopic observations.

In **brightfield microscopy**, objects are dark and the field is light. Brightfield microscopy can be used to observe unstained microorganisms in wet mounts and hanging drop slides.

Observing Living Microbes

In this exercise, you will examine, using wet-mount techniques, different environments to help you become aware of the numbers and varieties of microbes found in nature. The microbes will exhibit either Brownian movement or true motility. **Brownian movement is not true motility but rather movement caused by the molecules in the liquid striking an object and causing the object to bounce.** In Brownian movement, the particles and microorganisms all vibrate at about the same rate and maintain their relative positions. Motile microorganisms move from one position to another. Their movement appears more directed than Brownian movement, and occasionally the cells may spin or roll.

Many kinds of microbes, such as protozoa, algae, fungi, and bacteria, can be found in pond water and in infusions of organic matter. The microbial communities in environments are called **microbiomes**.

Van Leeuwenhoek made some of his discoveries using a peppercorn infusion similar to the one you will see in this exercise. Direct examination of living microorganisms is very useful in determining size, shape, and movement. **Using a wet mount is a fast way to**

observe bacteria, but motility is difficult to determine because of the movement of the water. Motility and larger microbes are more easily observed in the greater depth provided by a hanging drop. Using a petroleum jelly seal reduces evaporation of the suspended drop offluid.

In this exercise, you will examine living microorganisms by brightfield microscopy.

Materials

Slides
Coverslips
Hanging-drop (depression) slide
Petroleum jelly
Toothpick
Pasteur pipettes
Alcohol
Gram's iodine
Inoculating loop

Cultures

Hay infusion, incubated 1 week in light
Hay infusion, incubated 1 week in dark
Peppercorn infusion
18- to 24-hour-old broth culture of *Bacillus*

Techniques Required

Compound light microscopy (Exercise 1)

PROCEDURE

Wet-Mount Technique

1. Suspend the infusions by stirring or shaking them carefully. Using a Pasteur pipette, transfer a very small drop of one hay infusion to a slide, or transfer a loopful using the inoculating loop, as demonstrated by your instructor.
2. Handle the coverslip carefully by its edges, and place it on the drop.

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A Gutmann



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