

Examination of Living Microorganisms

EXERCISE

2



MICROBIOLOGY

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



After demonstrating safety in properly preparing slides for microscopical examination including performing wet-mount slides or hanging-drop preparations and executing microscope 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 of fluid.

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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Arthur James Wells

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1. Understanding the eBook Medgar Microbio Lab Manual
 - The Rise of Digital Reading Medgar Microbio Lab Manual
 - Advantages of eBooks Over Traditional Books
2. Identifying Medgar Microbio Lab Manual
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Medgar Microbio Lab Manual
 - User-Friendly Interface
4. Exploring eBook Recommendations from Medgar Microbio Lab Manual
 - Personalized Recommendations
 - Medgar Microbio Lab Manual User Reviews and Ratings
 - Medgar Microbio Lab Manual and Bestseller Lists
5. Accessing Medgar Microbio Lab Manual Free and Paid eBooks
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 - Medgar Microbio Lab Manual Enhanced eBook Features
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 - Adjustable Fonts and Text Sizes of Medgar Microbio Lab Manual
 - Highlighting and Note-Taking Medgar Microbio Lab Manual
 - Interactive Elements Medgar Microbio Lab Manual
8. Staying Engaged with Medgar Microbio Lab Manual
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 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Medgar Microbio Lab Manual
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 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Medgar Microbio Lab Manual
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Medgar Microbio Lab Manual
 - Setting Reading Goals Medgar Microbio Lab Manual
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Medgar Microbio Lab Manual
 - Fact-Checking eBook Content of Medgar Microbio Lab Manual
 - Distinguishing Credible Sources
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 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
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