Negative Staining

LEARNING OBJECTIVES

Once you have completed this experiment, you should be able to

1. Perform a negative staining procedure.
2. Understand the benefit obtained from visualizing unstained microorganisms.

CLINICAL APPLICATION

Detecting Encapsulated Invaders

The principle application of negative staining is to determine if an organism possesses a capsule (a gelatinous outer layer that makes the microorganism more virulent), although it can also be used to demonstrate spore formation. The technique is frequently used in the identification of fungi such as *Cryptococcus neoformans,* an important infectious agent found in bird dropping that is linked to meningeal and lung infections in humans.

Principle

Negative staining requires the use of an acidic stain such as India ink or nigrosin. The acidic stain, with its negatively charged chromogen, will not penetrate the cells because of the negative charge on the surface of bacteria. Therefore, the unstained cells are easily discernible against the colored background.

The practical application of negative staining is twofold. First, since heat fixation is not required and the cells are not subjected to the distorting effects of chemicals and heat, their natural size and shape can be seen. Second, it is possible to observe bacteria that are difficult to stain, such as some spirilla. Because heat fixation is not done during the staining process, keep in mind that the organisms are not killed and *slides should be handled with care.* Figure 1 shows a negative stain of bacilli.

Figure 1 **Negative staining: *Bacilli* (1000 ×)**

AT THE BENCH

Materials

Cultures

24-hour agar slant cultures of *Micrococcus luteus*, *Bacillus cereus*, and *Aquaspirillum itersonii.*

Reagent

Nigrosin.

Equipment

Bunsen burner, inoculating loop, staining tray, glass slides, lens paper, and microscope.

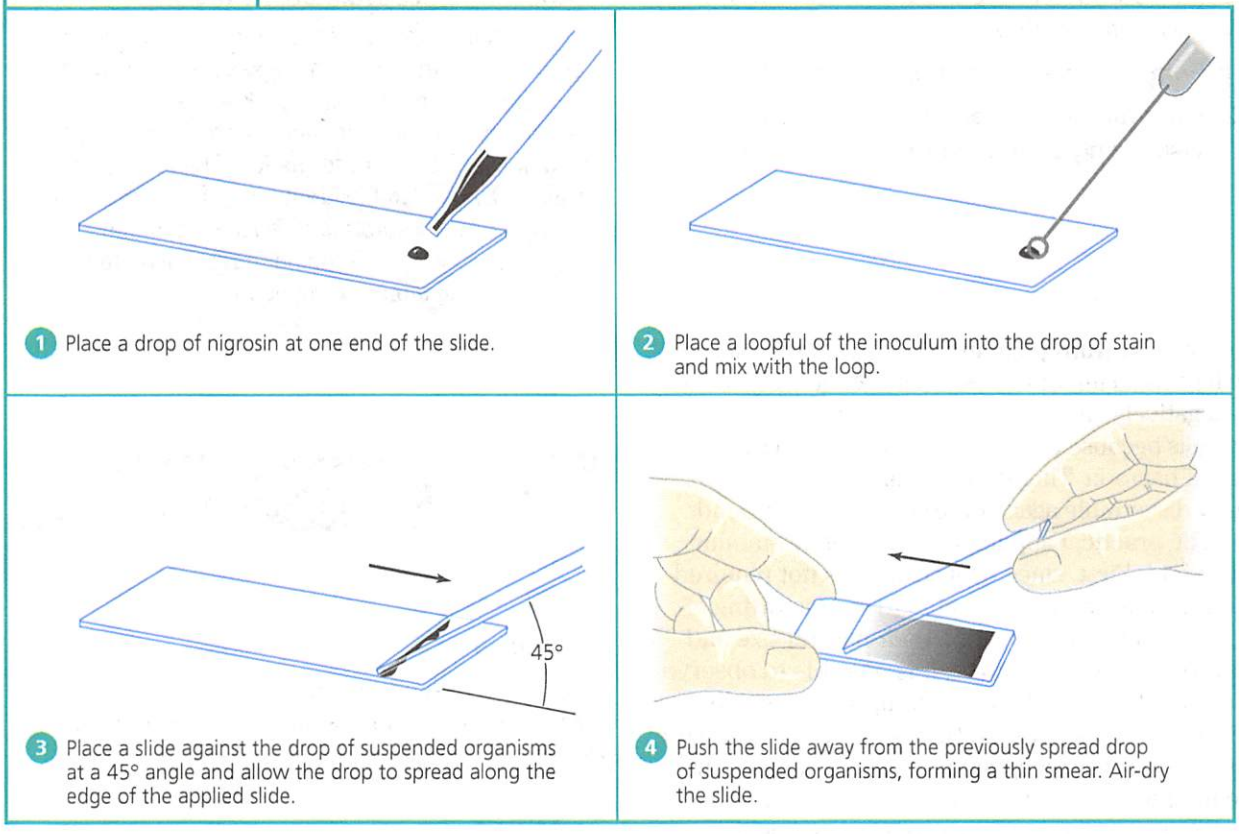
Procedure

Steps 1–4 are illustrated in Figure 2.

1. Place a small drop of nigrosin close to one end of a clean slide.
2. Using aseptic technique, place a loopful of inoculum from the *M. luteus* culture in the drop of nigrosin and mix.

3. Place a slide against the drop of suspended organisms at a 45° angle and allow the drop to spread along the edge of the applied slide.
4. Push the slide away from the drop of suspended organisms to form a thin smear. Air-dry. *Note: Do not heat fix the slide.*
5. Repeat Steps 1–4 for slide preparations of *B. cereus* and *A. itersonii*.
6. Examine the slides under oil immersion, and record your observations in the Lab Report.

PROCEDURE



1. Place a drop of nigrosin at one end of the slide.
2. Place a loopful of the inoculum into the drop of stain and mix with the loop.
3. Place a slide against the drop of suspended organisms at a 45° angle and allow the drop to spread along the edge of the applied slide.
4. Push the slide away from the previously spread drop of suspended organisms, forming a thin smear. Air-dry the slide.

Figure 2 **Negative staining procedure**

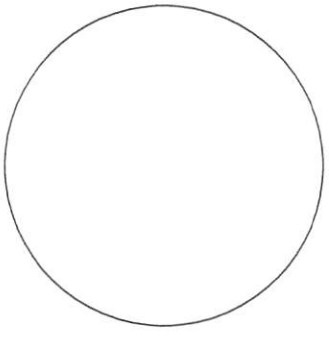
Name: _____

Date: _____ Section: _____

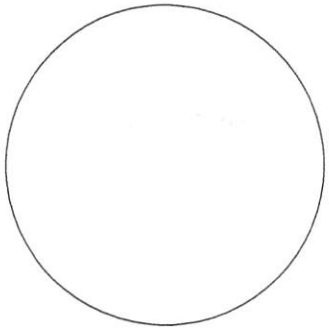
Lab Report

Observations and Results

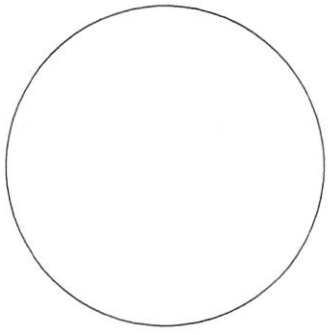
1. Draw representative fields of your microscopic observations.



M. luteus



B. cereus



A. itersonii

2. Describe the microscopic appearance of the different bacteria using the chart below.

Organism	***M. luteus***	***B. cereus***	***A. itersonii***
Shape			
Arrangement			
Magnification			

Review Questions

1. Why can't methylene blue be used in place of nigrosin for negative staining? Explain.

2. What are the practical advantages of negative staining?

3. Why doesn't nigrosin penetrate bacterial cells?

Photo Credit

Credits are listed in order of appearance.

Photo 1: Experiment performed and photographed by L. Brent Selinger, Department of Biological Sciences, University of Lethbridge, Pearson Education