

Exercise  
3-5

## Negative Stain

## MATERIALS

Nigrosin stain or India Ink

Clean glass microscope slides

Disposable latex gloves

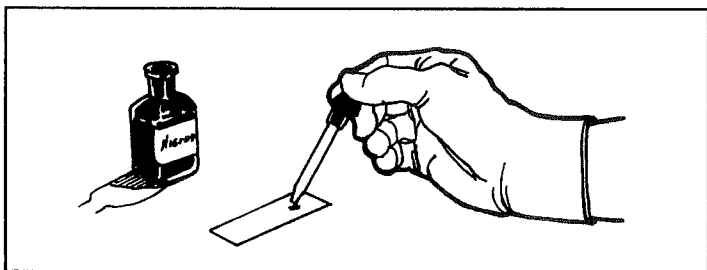
Recommended organisms:

*Micrococcus luteus**Bacillus megaterium*

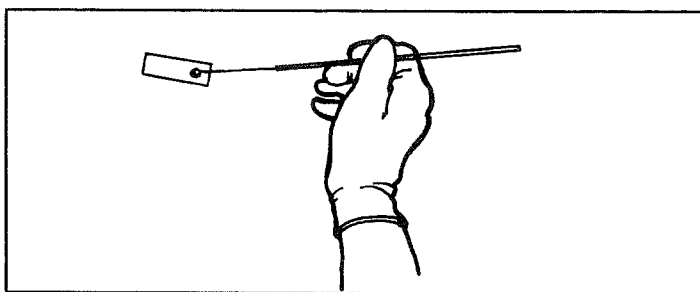
## STAINING PROTOCOL

Photographic Atlas  
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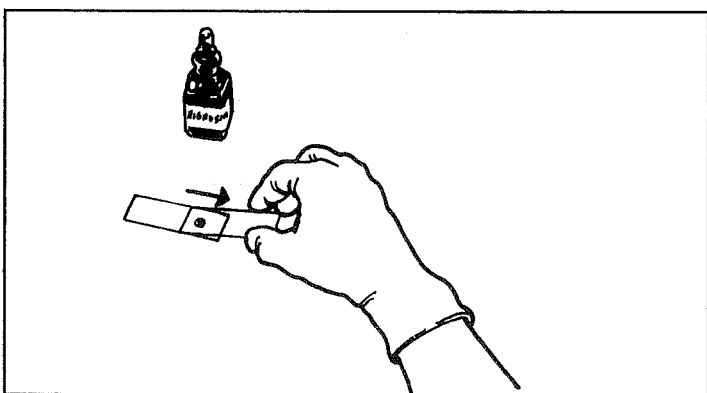
1. Figures 3-7a through 3-7e illustrate the procedure for preparing a negative stain.
2. Observe using the oil immersion lens. Record your results in the table on the following page.

**FIGURE 3-7a** Begin with the Stain

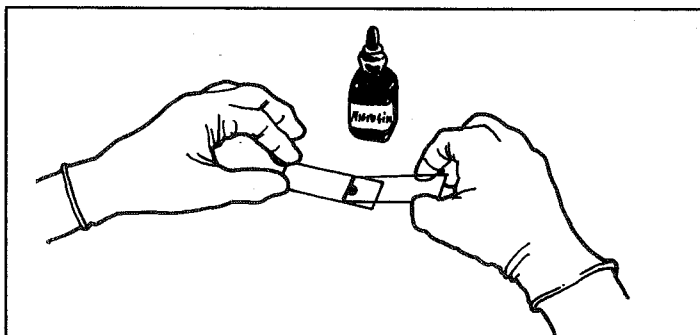
Place a small drop of nigrosin stain at one end of a clean glass slide. Avoid excess nigrosin on the slide. It is advisable to wear latex gloves to protect your hands.

**FIGURE 3-7b** Add the Organism

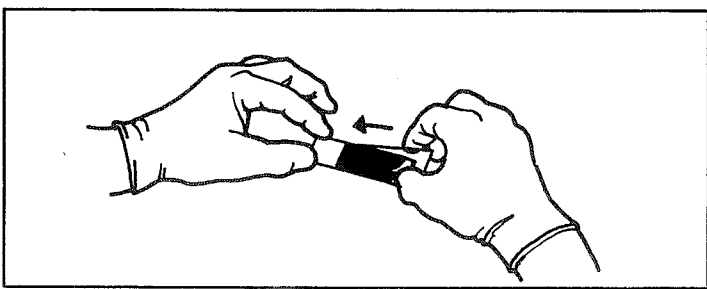
Use a loop to aseptically transfer cells to the slide. Gently mix the organisms into the nigrosin. Avoid over-inoculating the slide or spattering the contaminated nigrosin drop as you mix. Flame the loop before proceeding.

**FIGURE 3-7c** Use a Second Slide as a Spreader

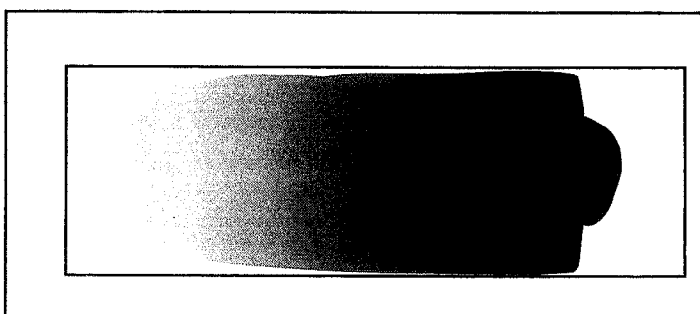
Place a clean second slide on the surface of the first slide and carefully back it up into the drop of nigrosin.

**FIGURE 3-7d** Not Too Far!

As soon as the nigrosin flows across the width of the spreader slide, stop.

**FIGURE 3-7e** Spread a Nigrosin Film Across the Slide

Make a nigrosin smear by pushing the spreader slide across the specimen slide's surface. Allow the film to air dry. Dispose of the spreader slide appropriately since it is contaminated.

**FIGURE 3-7f** The Finished Product

After air drying, the slide should look like this. Use the oil lens to observe a region where the nigrosin is relatively thin.