

## Preparing a Bacterial Smear on Microscope Slides:

### Examining a Simple Stained Specimen Microscopically

#### Introduction

Bacteria generally take several days to grow on media which will allow for its/their presumptive identification[s]. Clinicians need to know fairly quickly in general terms what type of bacteria they are trying to treat in individuals presenting with bacterial diseases. The fastest way in which to get this kind of information is to prepare a microscope slide with specimen on it followed by a specific kind of staining. Based upon the reaction of the bacteria to/with the stains, the clinician is then able to select a broad spectrum antibiotic and begin antibiotic therapy for the patient. This approach is sometimes known as the "shotgun" approach.

This experiment will take you through the steps of a general microscope slide/bacterial sample preparation and then on to a simple staining method for the visualization of the bacteria. (Remember that bacteria are very difficult to see without stains.)

#### Materials and Methods

##### *Materials*

Inoculated Agar Plate	Striker	Bunsen burner
Disinfectant	Microscope slide (1)	Wax marking pencil
Distilled water	Methylene blue and staining rack	Paper towels
Microscope	Bibulous paper	Lens paper
Paper towels	Immersion oil	Colored pencils

##### *Method*

#### Slide Preparation

Obtain 1 microscope slide and a wax marking pencil. In the center, more or less, of the slide, make a target circle about the size of a nickel. Flame your loop and

use the loop to place one to two drops of water in the target circle of each slide. Reflame the loop and allow it to cool. Remove one colony of bacteria from one of the plates and place it in one of the target circles and smear it around in the water. Allow your slide to completely air dry.

Once the slide is air dry, carefully run it through your flame (as demonstrated by your professor) until the slides are about the temperature of baby's milk to your wrist. This process "fixes" the bacteria to the slides. This heat (1) kills the bacteria and (2) makes the bacteria stick to the slide so you may stain it without losing your sample.

### Staining Method

Place your slide on your staining rack. Pour methylene blue on the target circle of your slide. Let your slide set for 1 minute, then rinse off the stain with tap water. Dry the slide with bibulous paper (your instructor will demonstrate this process).

All slides need to be examined under the microscope using the immersion oil and the immersion oil objective (100X objective). Remember to begin focusing with the 10X (low power objective) and the coarse focus adjust. Continue on to the 40X (high, dry objective) using the fine focus adjust. Once the sample is focused on the 40X objective, turn the nose-piece half-way between the 40X and the 100X objectives, LEAVE THE STAGE ALONE, place one drop of immersion oil on the microscope slide where the light is coming through, then swing the 100X objective right into the oil. Use ONLY the fine focus adjust knob from this point on (i.e., on the 100X objective). Record what you see (ask for assistance if you need it) in the spaces below:

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## REFERENCES

1. Beishir, L.: **Microbiology in Practice: A Self-Instructional Course, Fifth Edition.** (Harper Collins: New York) ©1991.
2. Gardner, P. and Provine, H.T.: **Manual of Acute Bacterial Infections: Early Diagnosis and Treatment.** (Little, Brown and Co.: Boston) ©1975.