Microbiology Simple Stain & Wet Prep Procedures

Objective:

• Learn how to prepare a simple stain and a wet preparation in which to visualize the microscopic morphology and arrangement in the microbial world.

Materials required:

- Glass slides, clean and Coverslips
- 0.85%Saline
- Disposable pipettes
- Methylene Blue
- Microscope drawing forms
- Specimens: 18-24 hour yeast culture (Saccharomyces cerevesiae), Escherichia coli, Staphylococcus aureus and Mouth swab/saliva

Simple stain from broth/saline: (Information only)

- 1. Using a sterile pipette place a small drop of the specimen in the center of a clean glass slide.
- 2. Using the tip of the pipette spread the drop to about quarter size.
- 3. Add a small drop of methylene blue. Mix using the tip of your pipette.
- 4. Carefully place a clean coverslip over the drop.

Simple stain from plate (WET PREP):

- 1. Place a small drop of saline in the center of the clean glass slide.
- 2. Using a sterile bacteriological loop touch a bacteria colony. Do not pick up the whole colony.
- 3. Place the loop into the drop and mix the bacteria and saline. Spread to about quarter size.
- 4. Add a small drop of methylene blue. Mix using a sterile loop.
- 5. Carefully place a clean coverslip over the drop.

Hints: This is a wet preparation. The techniques for a wet prep apply.

Simple stain from plate (STAIN):

- 1. Follow steps 1-3 from Wet Prep Procedure
- 2. Heat fix specimen to cause the specimen to adhere to the slide. (Watch demonstration)

Examination:

 Using a properly set up microscope, observe the wet preparations under low (100X) and high (400X) powers. Observe cell morphologies and organization. Using correct prospective and scale, draw what you see and properly label the drawing. Minimally, include labels identifying magnification and subject. For observations, describe the cells seen. (1 pt).

Organism	Stain & duration	Cellular Morphology & Arrangement (Include a sketch)

- 2. What is the advantage of using a stain versus not using one? (1 pt).
- 3. What is the consequence of leaving a stain on the bacterial smear too long (overstaining)? (1 pt).
- 4. What is the consequence of not leaving a stain on the bacterial smear long enough (understaining)? (1 pt).
- What is an advantage of doing a Wet Prep versus a simple stain, i.e. what can one detect doing the Wet Prep that one cannot detect doing a stain? (1 pt).