Clinical Bacteriology II

CLS 413

**Bacterial Smear and Gram Stain**

**Preparation and Heat Fixation of a Bacterial Smear**

1. Placing the bacteria on the slide:
2. You are provided with a bacterial culture plate from which you will prepare a smear.
3. Label your slide with a pencil (to be able to know which face is up).
4. Place a drop of water or saline on a glass microscope slide using a Pasteur pipette
5. Using a sterilized and cooled inoculation loop, obtain a very small sample of a bacterial colony.
6. Gently mix the bacteria into the saline drop
7. Heat Fixing the Bacterial Sample:
8. Let the bacterial smear air-dry for a few minutes (at least 5 min)
9. Place the slide on the hot plate or on top of the incinerator to be heat fixed.
10. Once the slide is heat fixed, proceed to gram staining.

**Gram Staining**

1. Flood the fixed smear with crystal violet solution. Allow to remain for 1 minute.
2. Rinse off the crystal violet with distilled or tap water.
3. Flood the slide with iodine solution. Allow to remain for one minute.
4. Rinse off the iodine solution with distilled or tap water.
5. Flood the slide with decolorizer for one to five seconds.
6. Rinse off the decolorizer with distilled or tap water.
7. Flood the slide with safranin. Allow to remain for 1 minute.
8. Rinse off the safranin with distilled or tap water.
9. Dry the slide on filter paper or absorbent paper and place in an upright position.
10. Microscopically examine the slide for bacterial organisms under a 100X objective.

\*Describe the gram reaction of any organisms seen.