Introduction to Microbiological Microscopy:

A Smattering of Stained Bacteriological Specimens

Introduction

Bacteria are very difficult, if not impossible, to visualize without some kind of stain. The stain, depending on its characteristics, and the cell, depending on its characteristics, determine what the final appearance of the stained cell will be.

India ink or Black Nigrosin, for example, stain everything BUT the bacteria black. When one of these dyes is used, this is called, appropriately enough, a background stain. Bacteria which respond to crystal violet and Gram's iodine are purple and are called Gram positive bacteria. Those bacteria which are decolorized by the action of acetone:alcohol and then bind safranin are pinkish-orange and are called Gram negative bacteria. Bacteria which bind a mixture of carbol fuchsin are called acid fast bacteria and are a brilliant fuchsin. Those bacteria which are decolorized by the action of acid:alcohol and bind methylene blue are called non-acid-fast and are blue. Bacteria which bind crystal violet and are surrounded by a clear zone which is surrounded by a fuchsia zone have been capsule stained. The clear region is the capsule (observed much like a photograph negative).

Bacteria also come in many different sizes and shapes. Cocci are spherical bacteria and look like very distinct "dots". Rods, a.k.a. bacilli, are many-shaped. While most are, indeed, rod-shaped, many are curved like a comma and some appear to be a hybrid between cocci and rods. The latter group are called coccobacilli. It takes much practice to get the shape differences correct. Other microbes are long and cylindrical and look like a corkscrew. These are called spirochetes.

The function of this lab is twofold:

1) To remind you of the working of the microscope, and

2) To let you observe virtually "perfect" slides of various sizes and shapes of microbes in anticipation of the slides each of you will be making in the future.

Materials and Methods

Materials

Prepared slides	Microscope	Immersion oil
K. pneumoniae	Bacilli	Gram negative cocci
S. pneumoniae	Gram positive rods	T. pallidum
S. pneumoniae	Gram negative rods	Gram positive cocci
Cocci	Lens paper	Drawing supplies

Methods

Always begin focusing the microscope on the low power objective (10X) with the stage in the lowest position and using the coarse adjust focusing knob. Once the specimen is in focus, turn the nosepiece so that the 40X objective (high, dry objective) is over the sample. REFRAIN FROM MOVING THE STAGE OR NOSE-PIECE AS THESE MICROSCOPES ARE **PARFOCAL**. Using the fine adjust focusing knob, focus your sample. WITHOUT MOVING THE STAGE, swing the nosepiece halfway between the 40X and 100X objective (oil immersion lens) and place one (1) drop of immersion oil onto the microscope slide right where the light is coming through the slide. Now, WITHOUT MOVING THE STAGE, swing the 100X objective right into the oil. Focus using ONLY the fine focus adjust knob until the specimen is clearly focused. Draw what you see in the provided spaces below. Repeat the process for all 10 of the prepared slides.

When you have completed the examinations, remove the immersion oil from your slides and from the 100X objective with lens paper, as well. Although there ought not to be any oil on any of the other objectives, please wipe them off as they may have inadvertently been "dunked".

REFERENCES

1.Beishir, L.: **Microbiology in Practice: A Self-Instructional Laboratory Course, Fifth Edition**. (Harper Collins: New York) ©1991.

2.Thomas, C.G.A.: **Medical Microbiology, Sixth Edition**. (Bailliere Tindall: London) © 1989.