LAB WORK 4.

Subject: Staining Methods. Simple Staining with Methylene Blue.

Session Purpose: Learn of how to make a Simple Staining with Methylene Blue. How to Prepare & Heat Fix a Microbial Smear for Staining.

Objectives:

1. Learn of how to make a stainedvital"crushed drop".
2. Learn of how to make a smear using the heat fixation process.
3. Learn of how to make a stainedfixed smear.
4. Performexercises 1, 2.

Not all specimens can be clearly seen under a microscope. Sometimes the specimen blends with other objects in the background because they absorb and reflect approximately the same light waves. You can enhance the appearance of a specimen by using a stain. A stain is used to contrast the specimen from the background.

A stain is a chemical that adheres to structures of the microorganism and in effect dyes the microorganism so the microorganism can be easily seen under a microscope. Stains used in microbiology are either basic or acidic.

Basic stains are cationic and have positive charge. Common basic stains are methylene blue, crystal violet, safranin, and malachite green. These are ideal for staining chromosomes and the cell membranes of many bacteria.

Acid stains are anionic and have a negative charge. Common acidic stains are eosin and picric acid. Acidic stains are used to stain cytoplasmic material and organelles or inclusions.

There are two types of Stains: simple and differential. A simply stain has a single basic dye that is used to show shapes of cells and the structures within a cell. Methylene blue, safranin, carbolfuchsin and crystal violet are common simple stains that are found in most microbiology laboratories.

There are two ways to prepare a specimen to be observed under a light compound microscope. These are a **wet mount** and a **smear**.

A **wet mount** is a preparation process where a live specimen in culture fluid is placed on a concave glass side or a plain glass slide. The microorganism is free to move about within the fluid, although the viscosity of the substance slows its movement. This makes it easier for you to observe the microorganism. The specimen and the substance are protected from spillage and outside contaminates by a glass cover that is placed over the concave portion of the slide.

Obviously if trouble has been taken to isolate pure cultures of bacteria, one wants to ensure that only these bacteria are viewed under the microscope.Glass slides are not sterile and are often coated with numerous bacteria as well as dust and spray cellulose fibres. Slides should therefore be wiped clean with tissue paper and passed once or twice through a bunsen flame. Cover slips when used should be wiped with tissue paper. Always hold slides either in a slide holder or on the edge surfaces not on the planar surface. Cover slips should always be held be the edge surface.

A **smear**is a preparation process where a specimen that is spread on a slide. You prepare a smear using the heat fixation process. For most staining procedures one need only air dry the film by holding the slide high above a bunsen flame, when dry pass the slide film side up, three times through the flame to kill and fix the cells. Too much heat will distort the shape of the organism and could alter its uptake of stain - the slide should feel warm but not hot. Bear in mind that staining procedures kill the cells and that some of the cells characteristics may inevitably be altered.All stainings are to be carried out over the staining rack or in the sinks.

The purpose of simple staining technique is to determine cell shape, size and arrangement of bacterial cells. It is a simplest staining technique to get knowledge about bacterial shape, is either coccus or bacillus or spiral in shape.

Lab Exercise 1.Vital"crushed drop"specimen - wet mount.

Methodical instructions: Prepare a stainedvital"crushed drop"specimen of agar microbial culture.Observe the cells of Yestsand describe what you see.

Procedure:

1. Obtain a clean microscope slide.
2.Place one drop of water on the slide.

3. Using a sterilized and cooled inoculation loop, obtain a very small sample of a bacterial colony.
4. Gently mix the bacteria into the water drop.
5. Place one drop of a weak solution (1:1000) dye (methylene dark blue or fuchsin).
6. Hold the side edges of the coverslip and place the bottom edge on the slide near the drop of specimen.

7. Slowly lower the coverslip into place.

8.View the microorganism with the high-power (x40) objective.
9. Sketch a picture of the microorganism.
10. Sign the pictureand specify Total Magnification (TM).

Lab Exercise 2.Heat Fixing the Microbial Sample.Stainedfixed smear.

Before staining, the sample must be heat fixed. This process accomplishes three things. Itfunctionsto:

- killthebacteria
- firmly affix the smear to the microscope slide
- allow the sample to more readily take up the stain.

In order to heat fix a bacterial smear, it is necessary to first let the bacterial sample air dry. Then either place the slide in the slide holder of a microincinerator,or pass the dried slide through the flame of a Bunsen burner 3 or 4 times, smear side facing up. Once the slide is heat fixed, it can then be stained.

Methodical instructions: Prepare a stainedfixed smear of agar microbial culture.To go for Simple staining the first thing you should have to do, is to prepare a thin bacterial film i.e. Bacterial smear on a clean and dry glass slide and fix it. Make it by mixing a small amount of Microbial culture in small water droplet and spread it. Just fix it by heating carefully. You can follow the part two of this series to make Microbial smears perfectly and quickly fixed.

Application of staining reagent (dye). When your smear is ready go with any basic dye (cationic dyes e.g. crystal violet, carbolfuchsin, methylene blue etc.) and flood it on heat fixed Microbial smear for few seconds. Different dyes have different exposures time to stain cell,

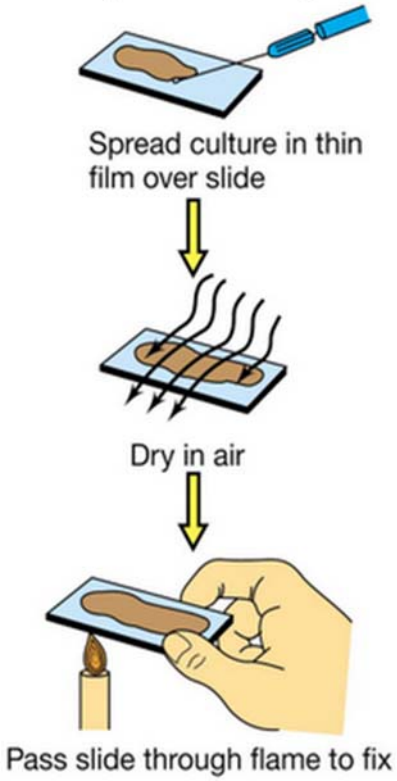
for example-crystal violet,2-60 seconds; carbol fuchsin,15-30 seconds and methylene blue,15-20 seconds. The increased time will result in increase in darkness.

Removal of excess dye and observation. You applied the stain, some amount of which get bound to the cells while some amount is freely moving in solution. To eliminate or say minimize the background noise of stains it is necessary to Remove remaining amount. It is simply done by washing out the slide with water and then dry it either in air or by blotting the surface now apply a drop of oil-immersion on it and examine under 100X of microscope to see colourful cells under transparent background.

Procedure:

a) Heat Fixing the Microbial Sample.

1. Use a clean glass slide.
2. Take a loop of the culture.
3. Place the live microorganism on the glass slide.
4. The slide is air dried then passed over a Bunsen burner about three times. The heat causes the microorganism to adhere to the glass slide. This is known as fixing the microorganism to the glass slide.



b) Stain the microorganism with an appropriate stain:

1. Place a slide on the centre supports of the staining rack, and flood the smear with a few drops of the methylene blue stain, and allow to act for 1-3 min.
2. Wash the smear with water (either from a wash bottle or a slow running tap) to remove dye.
3. Dry the slide using absorbent paper pressed lightly over the surface.
4. Examine the stained preparation under the x40 objective.
5 .Sketch a picture of the microorganism.
6. Sign the pictureand specify Total Magnification (TM).

Flood slide with stain;
rinse and dry

Notice: No coverslip is required with stained preparations, but take due care when using high magnification that the objective lens does not touch the smear.Proper Storage of Your Microscope.

Equipment:

- Microscope
- Slide
- Coverslip
- Dropper
- Dropper bottle of water
- Disinfectant tray
- Culture of Yeasts in slant tubes
- Inoculation loop
- Burnerflame
- Staining material - Methylene blue stain