

## Practical No. 5

### Bacteriological Stains and Staining Procedures;

#### 1- Types of stains

#### 2- Studying cellular Morphology

In order to study the bacterial cellular morphology; shape, size, length, diameter, as well as aggregation, it should be seen clearly under the microscope with high magnification power. Since this is difficult to be applied using fresh specimens, because the bacteria is bouncing in the liquid specimen or moving truly, even though using a cover slide, because the bacteria contains little constituents making them transparent or semi transparent. Therefore, in order to study cellular morphology and aggregation, bacteria should be killed, fixed and stained with the specific bacteriological stains.

There are two types of bacteriological stains used ;

- 1- **Basic stains;** the stain molecule in such type of stains possess positive charge after ionization, such as; crystal violet, methyl violet, methylene blue, safranin.....etc.
- 2- **Acidic stains;** In such stains, the stain molecule will be charged negative after ionization. Such as; India ink and nigrosin.

Since the bacteria contains specific proteins in their cell wall, as well as high content of ribonucleic acid make the bacteria possess negative charge, therefore, the basic stains will be attracted to the bacterial cell and positively stained and take the color of the stain used. On the other hand, the stain molecule of the acidic stains will be repelled from the bacterial cell and a clear zone around the cell will be seen.

There are three staining procedures applied depending on the purpose of use;

#### **A-Simple staining technique;**

This procedure is simple, easy, time saving and economic. It is applied by using only a single stain and all the bacteria in the specimen will be stained by one color. It gives an idea if there is bacteria in the sample or not, and also tells you the shape, size, amount of the bacteria present.

The procedure for simple staining is as follows;

- 1- Prepare a clean, grease-free slide, if not sure clean it with soap and water and dry it with a clean cloth. Then mark it, on one of its angles, with your initials and the specimen or organism used.
- 2- Put a small drop of tap water (or distilled water) in the center of the slide, if you are using a broth culture, there is no need to put the drop of water. Take a small colony, or part of a large colony by the loop, and suspend it by continuous circulation of the loop with the drop of water, without touching the edges of the slide, until a homogenous suspension is formed, do not make heavy suspension, try to get a one layer thick suspension, You should be careful, because the suspension is till now is highly contagious!

- 3- Leave to dry at room temperature away from any working area. Do not heat because it might damages the cells, or blow air at the suspension to avoid scattering of invisible droplets which might contaminate any item it settles on.
- 4- Hold the slide from one far edge, and pass it quickly enough, not too slowly which might damages cells, and not too fast which might be not enough to kill and fix the cells. This process will denaturate the cell's protein and fix them on the slide. The slide is now safe from contamination, and ready for staining.
- 5- Put the slide on a slide rack on the washing sink, and cover the film (smear) with any of the basic stains available, such as; crystal violet, methyl violet or others. Leave for one minute.
- 6- Wash with running water on the washing sink, and leave to dry in an angle position.
- 7- Examine under the microscope using the oil immersion lens.

*Exercise;* describe the organisms you see under your microscope.

### **B-Differential staining technique ( Gram stain);**

This was first applied by the Danish scientist Christian Gram in 1884, where by this procedure the bacteria are classified into two main groups, which are called; Gram positive, and Gram negative. This classification is very important in identification and characterization of bacteria, since each group has its specific biochemical and physiological characters.

#### **Procedure for staining:**

- 1- Prepare a fixed smear by following the steps 1 – 4 as in simple staining.
- 2- Cover the smear with crystal violet for 1 minute.
- 3- Wash with water
- 4- Cover with Gram iodine ( KI + I) for 1 minute. The stain now is combined with iodine forming what is called *Crystal Violet Iodine complex (CVI complex)*. This complex will be fixed in bacteria in its cell wall more than the stain alone. At this step all types of bacteria present in the specimen smear are colored by one color which is the dark violet color.
- 5- Wash with water and get rid of the excess water on the slide.

This step is a very critical step, where the decolorizing agent ( which is absolute alcohol, or absolute alcohol + acetone) will be used. The use of the decolorizing agent is only for few seconds (2 - 4 seconds) or longer, depends on the concentration of the alcohol and the acetone used. The group of bacteria that retain the color after using the alcohol will remain colored dark blue to violet and termed *Gram positive bacteria*. While the bacteria that lose its color after using the decolorizing agent will become colorless and later will be colored by the counter stain (red) and are termed *Gram negative bacteria*.

We called this step (very critical), because, if longer time than required is used, the color will be removed from all types of bacteria, and then all will take the color of the counter stain (i.e. red), on the other hand, if shorter time than required is used, the color will not be removed from any type of bacteria and all will possess the same color, which is the crystal violet color.

Gram negative bacteria, in general, possess large amount of lipids in their cell wall more than that in Gram positive group, the use of alcohol ( or alcohol + acetone) will dissolve these lipids making pores in the cell wall allowing the stain complex to pass through these pores and leave the cell much faster than what occurs in Gram positive group, this is called the Pore Theory.

- 6- Wash with running water immediately. Cover the smear with the counter stain which is safranin in order to color the bacteria that have lost the stain during the use of alcohol. Leave for 1 minute, and then wash with water.
- 7- Leave to dry at room temperature in an angle position.
- 8- Examine with oil immersion lens
- 9- Record your results, and draw what you see under the microscope.

### **Cellular Morphology;**

There are several forms or shapes of bacteria known. Besides, certain types of bacteria may show a phenomenon which is called pleomorphism, where the same species of bacteria may show different forms depending on the environmental conditions.

Such character is very important as a primary step for identification of the bacteria.

In this practical, try to see and draw in your notebook the following forms of bacteria;

*Staphylococci*

*Streptococci*

*Diplococci*

*Neisseria*

*Short bacilli*

*Long Bacilli*

*Coccobacilli*

*Spirochaete*