Microbiology I

Lab/4 :- Smear preparation and staining

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Lab/4 :- Smear preparation and staining (simple and differential stains):

Simple staining depends on the fact that bacteria differ chemically from their surroundings and thus can be stained to contrast with their environment. The stains are mostly salts (few of them are alkalines and acids),these salts contains of color ions that charging with positive and negative charges, these ions contains of groups carry the stain (chromophere) such as methylene

blue.

Methylene blue +CL ⟶ Methylene blue chloride

(Chromophere)

If the chrom-carriers are positive ion that mean the stain is alkaline, while if the chrom-carriers are negative ion that mean the stain is acidic.

The stain that stains the bacterial cells called direct stain. While the stain that stains the glass of slide called negative stain.

Bacterial smear preparation:-

In the case of the broth culture :-

Shake the culture tube and with an inoculation loope, transfer 1to2 loopfulls of bacteria to the center of the slide. Spread this out to about a 1/2 inch area disruption of cellular arrangement A satisfactory smear will allow examination of the typical cellular arrangement and isolated cells .

In case of the solid culture (slant or plate):-

Place loopfull of water in center of the slide with the inoculating needle , aseptically pick up a very small amount of culture and mix in to the drop of water. Allow the slide to air dry, or place it on a slide warmer .

Pass the slide through a burner flame 3times to heat-fix and kill the bacteria.

Staining

1-Simple staining:-

1. Place the fixed smears on a staining loop or rack over a sink or other suitable place.

2.Stain with any stain for 1 minutes.

3. Wash stain off slide with water for a few seconds.

4. Blot slide dry with bibulous paper.

5. Put the slide on the stage of microscope and begin with low power objective then high power objective lenses lastly with oil immersion objective lens .

2- Differential staining:-

A/ Grams Staining

In 1884, the Danish physician Christian Gram was trying to develop a staining procedure that would differentiate bacterial cells from eukaryotic nuclei in stained tissue sample. What resulted from his work is most important stain in bacteriology, the Gram stain. The Gram stain is an example of a differential stain. These staining reactions take advantage of the fact that cells or structures within cells display dissimilar staining reactions that can be distinguished by the use of different dyes. In the Gram stain, two kinds of cells, gram-positive and gram-negative, can be identified by their respective colors purple and red to pink after performing the staining method.

The Gram stain composed of:

1-Crystal violet (base or main stain): it's stain the G+ve bacteria with a blue to purple color.

2-Gram iodine (mordant iodine): that react with crystal violet to make the [Crystal violet-iodine complex] it's colored with purple black.

3-Ethanol 95% or acetone: it use to remove the first stain.

4-Safranin (counter stain): it's stain the G-ve bacteria with a pink to red color.

Procedure:

1-Prepare a bacterial smear.

2-Flood the smear with crystal violet and let for l-2 minutes.

3-Wash the slide with water .

4-Cover the slide with gram iodine and let for 1 minute.

5-Decolorize with 95% ethanol for 15 seconds.

6-Wash the slide with water.

Flood the smear with the counter stain (safranin)for 1-2 minutes.-7

8-Wash the slide with water.

9-Dry the slide with bibulous paper.

10-Examine under oil immersion (since the gram-positive bacteria stain with blue to purple while

gram-negative stain with pink to red color).

B- Acid fast stain (AFB)(Ziehl-Neelson staining)

Bacteria such as Mycobacterium and Nocardia have cell walls that contain a high lipid content.

One of the cell wall lipids is a waxy material called mycolic acid. This material is a complex lipid that is composed of fatty acids and fatty alcohols that have hydrocarbon chains up to 80 carbons in length. It significantly affects the staining properties of these bacteria and prevents them from being stained by many of the stains routinely used in microbiology. The acid-fast stain is an important diagnostic tool in the identification of Mycobacterium tuberculosis the causative agent of tuberculosis, and Mycobacterium leprae the bacterium that causes leprosy in humans. The facilitate staining of these bacteria, it is necessary to use techniques that make the cells more permeable to stain.

The Ziehl-Neelson stain composed of:

1-Carbol fuchsin solution (red color).

2-Ethanol 95% acidified with 20% HCL or H2SO4.

3-Methylene blue solustion.

Procedure:-

1-Prepare microbial smear fiom the sputum specimen & fixed it.

2-Flood the slide with carbol fuchsin stain then heat it on the Benson burner for 5minutes.

3-Wash the slide with water.

4-Decolorize with the acid- alcohol for l0-20seconds.

5-Wash the slide with water.

6-Add methylene blue for 30 seconds .

7-Wash the slide with water & dry it with bibulous paper.

8-Examine the prepared slide under the microscope