AL-Balqa'a Applied University

Al – Huson University College

Environmental Microbiology Lab

Experiment "1"

Student Name:

Title of Experiment: Preparation of bacterial smears and staining

Objective

1. Prepare bacterial smears for the microscopic visualization of bacteria.

2. The chemical basis for the simple and gram stain.

3. The procedure for differentiating between two principal groups of bacteria: gram positive and gram negative.

Introduction

Bacterial smears must be prepared prior to the execution of any of the staining techniques. Although not difficult, the technique requires adequate care by preparation of glass microscope slide, labeling of slides, preparation of smear, broth cultures, cultures from solid medium and finally heat fixation.

In simple staining, the bacterial smear is stained with a single reagent, which produces a distinctive contrast between the organism and its background. Differential staining requires the use of at least three chemical reagents (primary stain, decolorizing agent, counter stain) that are applied sequentially to a heat- fixed smear.

The most important differential stain used in bacteriology is the Gram stain; it divides bacterial cells into two major groups, gram-positive and gram-negative that is differences in chemical composition of bacterial cell walls. G positive cells have a thick layer, whereas the peptidoglycan layer in G negative cells is much thinner and surrounded by outer lipid-containing layers.

Materials required and Equipment

24-hr nutrient agar culture, glass microscope slides, Bunsen burner, inoculating loop, staining tray, bibulous paper, microscope.

*Reagents: Crystal violet, gram's iodine, 95% ethyl alcohol and safranin.

Result

After applying this experiment we observe by microscope about type of bacteria that uses and we note that is **gram negative, red, cocci (spherical in shape).**

Discussion

In the Gram stain, an insoluble crystal violet-iodine complex is formed inside the cell, and this complex is extracted by alcohol from gram-negative but not from gram-positive Bacteria. The alcohol dehydrates Gram_positive Bacteria, which have very thick cell walls consisting of several layers of peptidoglycan. This causes the pores in the walls to close, preventing the insoluble crystal violet-iodine complex from escaping. In gram-negative Bacteria, alcohol readily penetrates the lipid-rich outer layer, and the thin peptidoglycan layer also does not prevent solvent passage, thus, the crystal violet-iodine complex is easily removed.