Al- Mustaqbal University College

First stage.
Department of Optometry(Optics)

جامعة المستقبل الاهلي
مرحلة الاولى
قسم التقنيات البصرية

GRAM STAINING

LAB : 3

DR:ZAINAB WADDAH NASER

3/2/2022

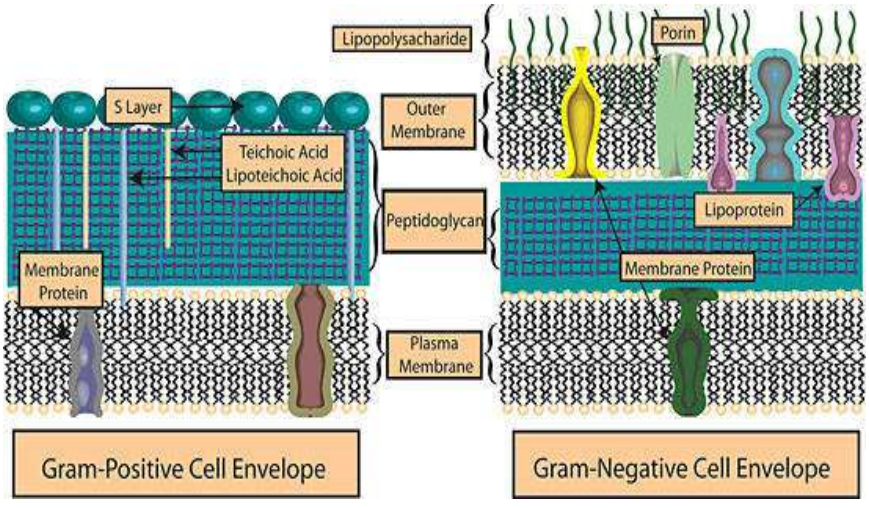
The Gram stain, the most widely used staining procedure in bacteriology, is a complex and differential staining procedure. Through a series of staining and decolorization steps, organisms in the Domain Bacteria are differentiated according to cell wall composition. Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan (90% of cell wall). These stain purple.

Gram-negative bacteria have walls with thin layers of peptidoglycan (10% of wall), and high lipid content. These stain pink. This staining procedure is not used for Archeae or Eukaryotes as both lack peptidoglycan. The performance of the Gram Stain on any sample requires four basic steps that include applying a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram's Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstaining with safranin.

The method is named after its inventor, the Danish scientist Hans Christian Gram (1853–1938), who developed the technique while working with Carl Friedländer in the morgue of the city hospital in Berlin in 1884. Gram devised his technique not for the purpose of distinguishing one type of bacterium from another but to make bacteria more visible in stained sections of lung tissue.

DIFFERENTIAL STAINING

Acceptance of stains is an important property of bacteria and is the base divission of bacteria to 2 principal groups Gram positive and Gram negative in taxonomy.



GRAM STAINING PROCEDURE

Prepare a heat fixed smear of the culture you wish to examine

Flood the smear with crystal violet (30 sec. to 2 min)

Quickly and gently wash off excess stain (2 seconds)

Flood the smear with Grams iodine (1 minute)

Decolorize with alcohol (10-20 seconds or until the excess alcohol which flow off the slide is colorless)

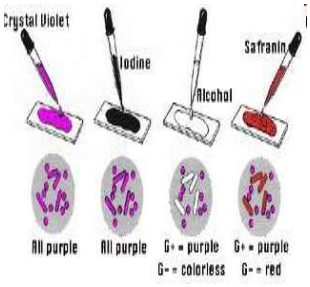
Quickly and gently wash off excess stain (2 seconds)

Flood the smear with safranin (carbolfuchsin) (30 sec to 2 min.)

Quickly and gently wash off excess stain (2 seconds)

Blot dry with bibulous paper

Examine your slide under the microscope. Record sketches of the organisms, size, color, morphology, and culture identification.



State why the gram stain is said to be a differential stain

Describe the differences between a gram-positive and a gram-negative cell wall

State the mechanism of the Gram stain and why differential staining of bacterial cells occurs.

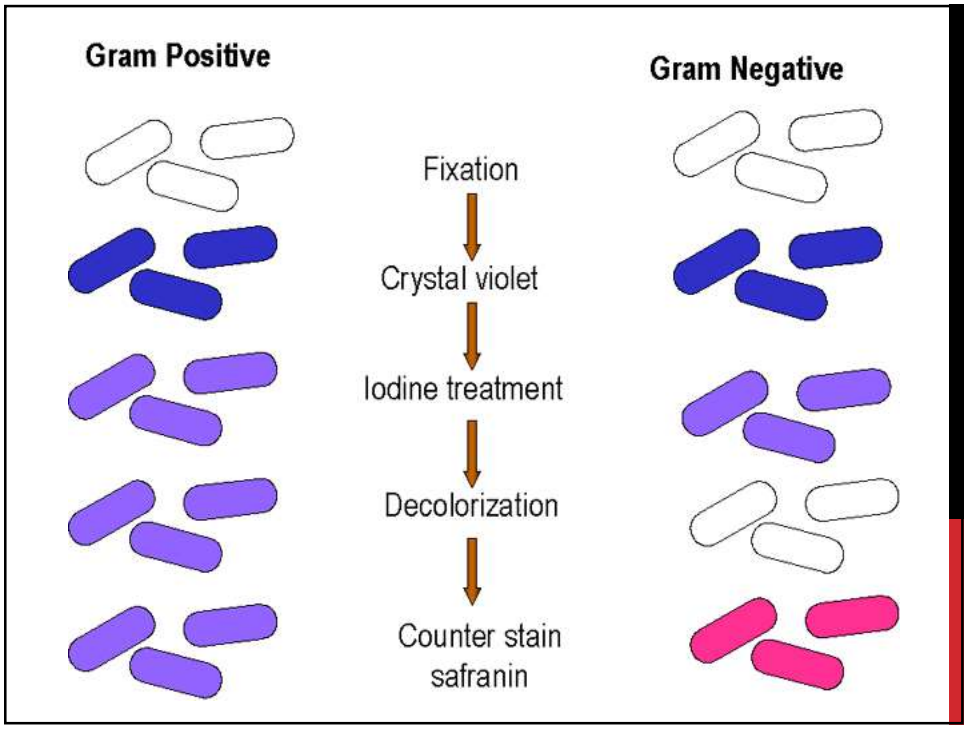
Have accurate labeled sketches of all of the specimens you examined. Included with the sketches should be a brief description of the specimen, the magnification under which it was viewed, and an approximation of the size.

Describe the conditions which may result in a gram-positive cell staining gram negative.

State the procedure for the gram stain

Perform a gram stain given all of the necessary materials and reagents

Determine if a bacterium is gram-positive or gram-negative when microscopically viewing the gram stain preparation and state the shape and arrangement of the organism.


Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin


CRYSTAL VIOLET
LUGOL
ACETON
CARBOLFUCHSIN

G+ G-