

Lecture 6

Gram stain

By:Suzan Hameed

introduction











- In 1884 the Danish bacteriologist Christian Gram developed a staining technique.
- Gram stain : is one of the most important techniques in microbiology
- It is a differential staining method used to classify bacteria into two major groups

- Gram-positive bacteria: Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan (90% of cell wall). These stain purple.
- Gram-negative bacteria: Gram-negative bacteria have walls with thin layers of peptidoglycan (10% of wall), and high lipid content. These stain pink .

- The procedure is based on ability of microorganisms to retain the purple color of crystal violet during decolorized by the alcohol
- Gram negative bacteria are decolorized by alcohol ,losing the purple color of crystal violet
- Gram positive bacteria are not decolorized and remain purple
- After decolorization , safranine, a red counterstain is used to impart a pink color to the decolorized gram negative organisms

- Crystal violet : primary stain cause both Gram negative and gram positive organisms to become purple after 20 seconds of staining.
- Gram's iodine: the mordant is applied to the cells for 1 minute, the color of Gram negative and gram positive bacteria remains the same (purple)
- The function of the mordant here is to combine with Crystal violet to form a relatively insoluble compound in the gram positive bacteria

- Decolorizing agent :95% ethanol is added to the cells for 10-20 seconds , the gram negative leached colorless, but the gram positive bacteria remain purple
- Final step a countersatoin safranine adds a pink color to the decolorized gram negative bacteria without affecting the color of the purple gram positive bacteria

REAGENT	GRAM-POS.	GRAM-NEG.
NONE (Heat-fixed Cells)		
CRYSTAL VIOLET (20 seconds)		
GRAM'S IODINE (1 minute)		
ETHYL ALCOHOL (10-20 seconds)		
SAFFRANIN (20 seconds)		

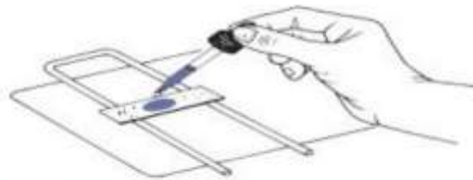
Note

- When working with unknowns keep in mind that old cultures of gram positive bacteria tend to decolorize more rapidly than young ones, causing them to appear gram negative instead of gram positive

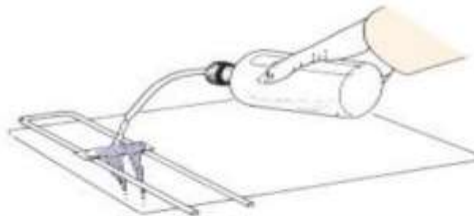
1. Cover the smear with **crystal violet** and let stand for *20 seconds*.
2. Briefly wash off the stain, using a wash bottle of distilled water. Drain off excess water.
3. Cover the smear with **Gram's iodine** solution and let it stand for *one minute*. (Your instructor may prefer only 30 seconds for this step.)
4. Pour off the Gram's iodine and flood the smear with **95% ethyl alcohol** for *10 to 20 seconds*. This step is critical. Thick smears will require more time than thin ones. *Decolorization has occurred when the solvent flows colorlessly from the slide.*
5. Stop action of the alcohol by rinsing the slide with water from wash bottle for a *few seconds*.
6. Cover the smear with **safranin** for *20 seconds*. (Some technicians prefer more time here.)
7. Wash gently for a few seconds, blot dry with bibulous paper, and air-dry.
8. Examine the slide under oil immersion.

ASSIGNMENTS

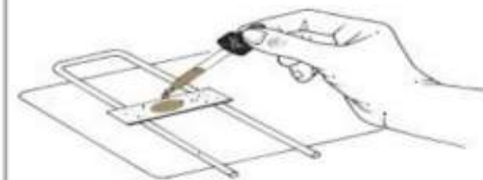
The organisms that will be used here for Gram staining represent a diversity of form and staining characteristics. Some of the rods and cocci are gram-positive;



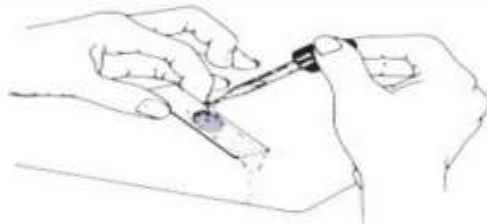
1 CRYSTAL VIOLET 20 seconds



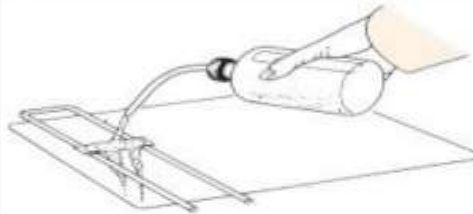
2 WASH 2 seconds



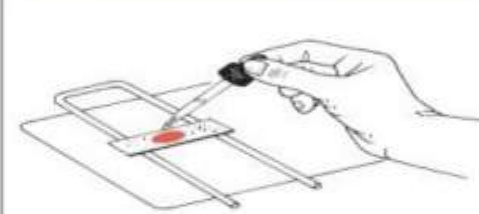
3 GRAM'S IODINE 1 minute



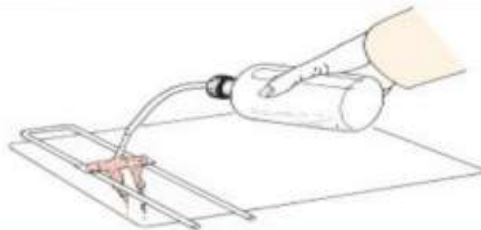
4 DECOLORIZE WITH ALCOHOL 10-20 seconds or until solvent flows colorlessly



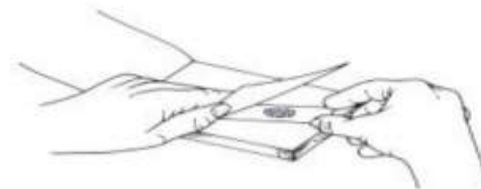
5 WASH 2 seconds



6 SAFRANIN 20 seconds



7 WASH 2 seconds



8 BLOT DRY

