BACTERIAL STAINING

TOPICS

- ✓ Types of staining techniques
- ✓ Gram Staining - Principles
- ✓ Gram Staining - Steps

Types of staining techniques

Simple staining
(use of a single stain)

For visualization of morphological shape & arrangement.

Differential staining
(use of two contrasting stains separated by a decolorizing agent)

Identification
- Gram stain
- Acid fast stain

Visualization of structure
- Spore stain
- Capsule stain

GRAM STAINING PRINCIPLES

- Gram reaction is based on the structure of the bacterial cell wall

Gram-positive bacteria

- The peptidoglycan appears to act as a permeability barrier preventing loss of crystal violet-iodine-complex.
- Purple

- When gram-positive bacteria are treated with alcohol, the alcohol causes coagulation and dehydration of the thick layer of peptidoglycan resulting in shrinkage of pores preventing CVI-complex from escaping and the bacteria remain deep colored.

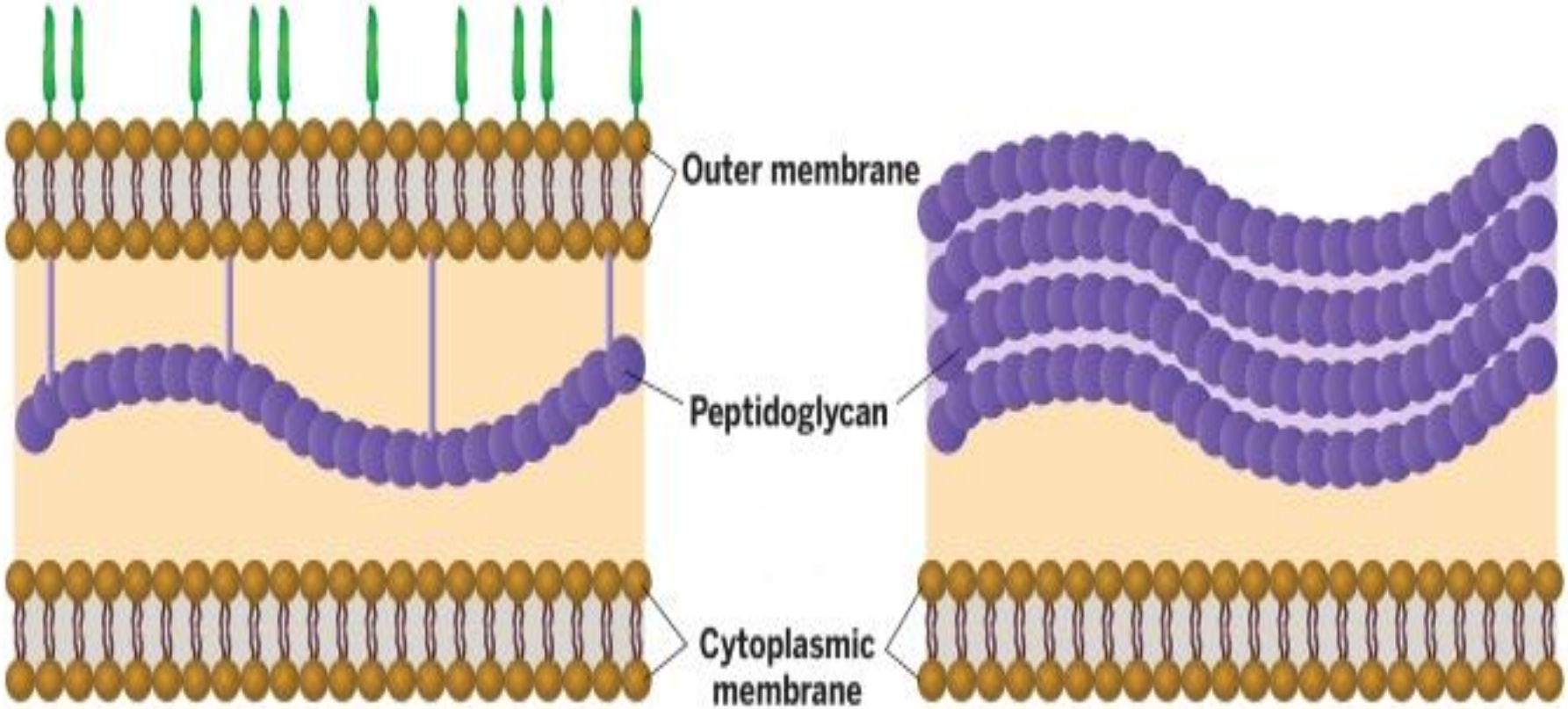
Gram Negative bacteria

- Peptidoglycan is very thin in gram (-) bacteria and has larger pores.
- Alcohol penetrates the lipid rich outer layer (LPS) of the cell wall and extracts enough lipid thus increasing the porosity further.

- Alcohol removes the deep purple CVI-complex from gram (-) bacteria thus becomes decolorized.
- The outer membrane is then permeabilized by the decolorizer, and the pink safranin counter stain is trapped by the peptidoglycan layer.

GRAM-NEGATIVE

GRAM-POSITIVE



GRAM STAINING STEPS

STEP 1: Make a smear and heat fixed.

STEP 2: Flood the entire slide with crystal violet (primary stain) for 1min. Then rinse with the water.

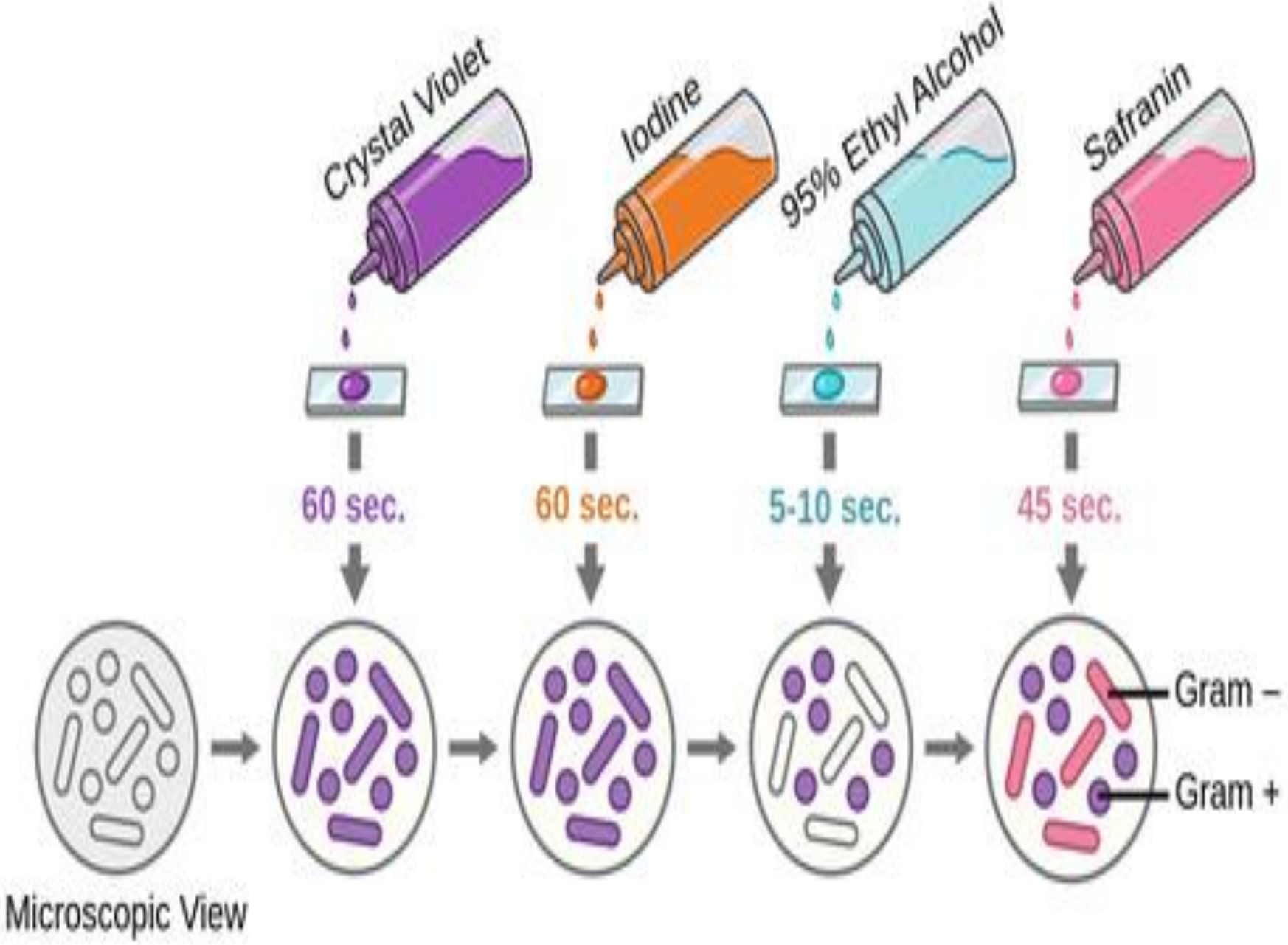
STEP 3: Flood the slide with the iodine solution (mordant) for 1min. Then rinse with water for 5 seconds. The bacteria become deeply stained and appear deep purple in color due to crystal violet-iodine-complex formation.

STEP 4: Addition of the decolorizer, 95% ethanol.

- Rinse with water.
- Gram (+) bacteria : purple dye is retained.
- Gram (-) bacteria : purple dye is readily removed and appears colorless.

STEP 5: Flood the slide with the counter stain safranin, then rinse with water.

- Gram (+) bacteria will remain purple in appearance.
- Gram (-) bacteria take on a pink/red color.


Crystal Violet
Iodine
95% Ethyl Alcohol
Safranin
60 sec.
60 sec.
5-10 sec.
45 sec.
Gram –
Gram +
Microscopic View

Differential Stains: Gram Stain

	Color of Gram + cells	Color of Gram – cells
Primary stain: Crystal violet	Purple	Purple
Mordant: Iodine	Purple	Purple
Decolorizing agent: Alcohol-acetone	Purple	Colorless
Counterstain: Safranin	Purple	Red

Organizing the Staining Bottles


1


4


Water
7


2


Water
5


Safranin
8


Water
3


6


Water
9

Gm+ve cocci & Gm-ve bacilli