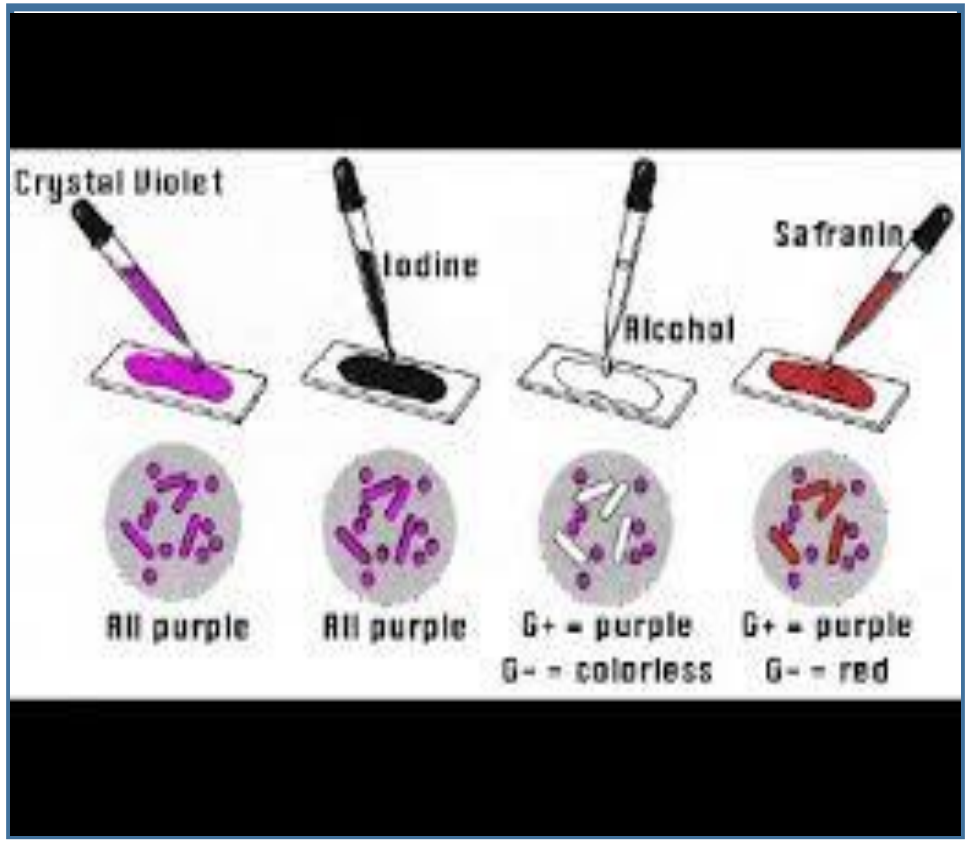


# Staining methods

# Lab (4)

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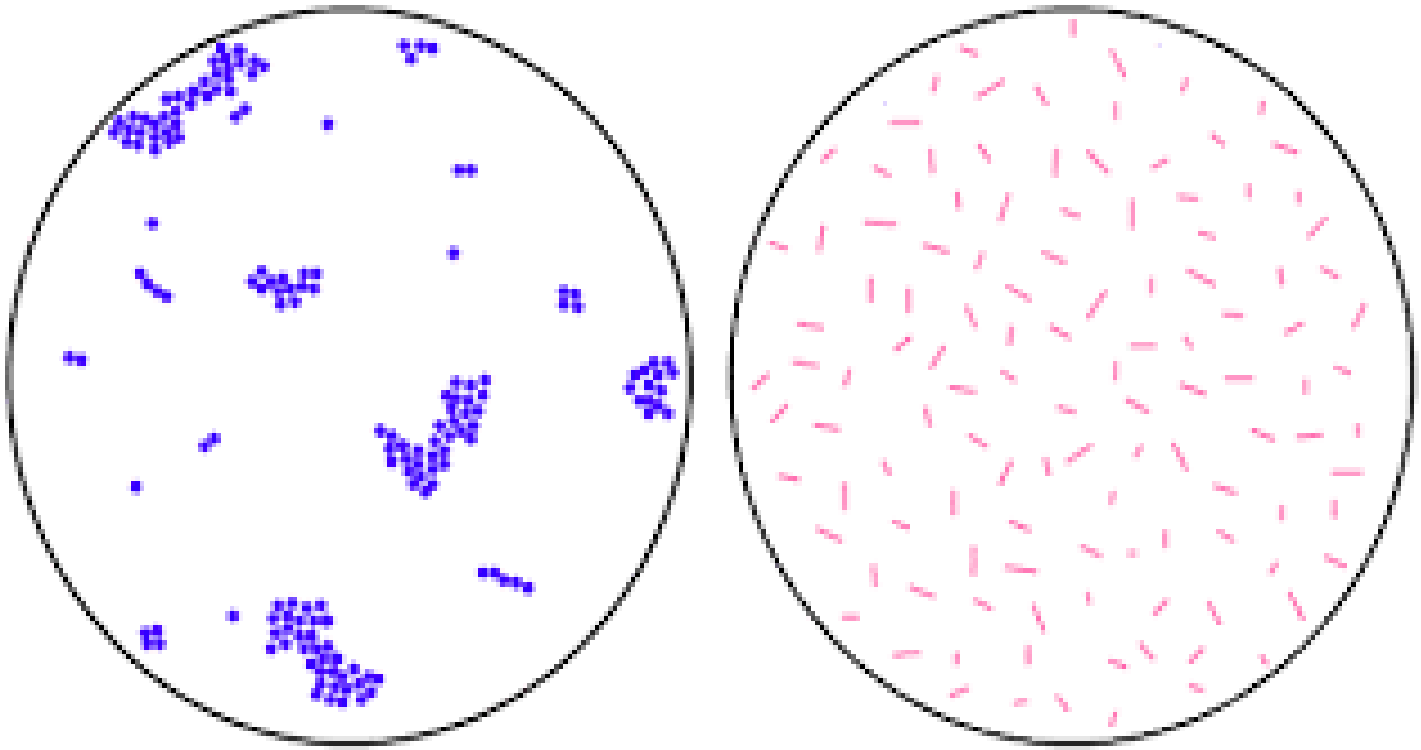
- **Why we stain bacteria**

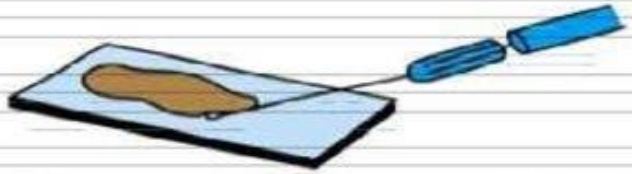
- Bacterial organisms are so small that most of them are visible under a microscope with a magnification power of 1000X. However, magnification of size does not provide a sufficient degree of clarity, so that bacteria must therefore be stained before observation to provide the clarity needed for visualization.
- The main purpose of staining is visualisation of bacteria.

# Simple staining

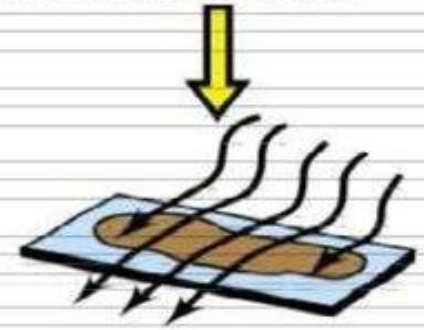
**simple stain** is a very **simple staining** procedure involving the use of only one **stain** such as methylene blue, Gram safranin, and Gram crystal violet.



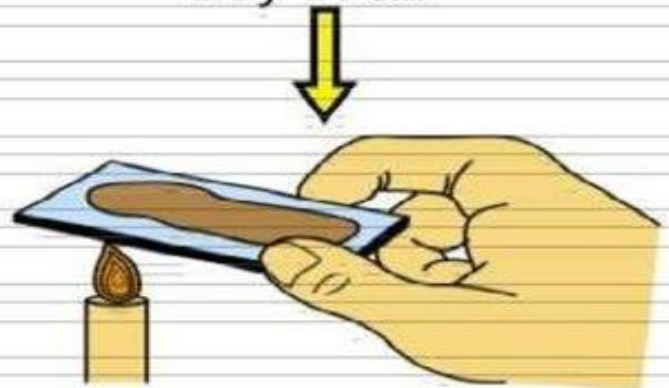
# Summary of simple stain



Spread culture in thin film over slide

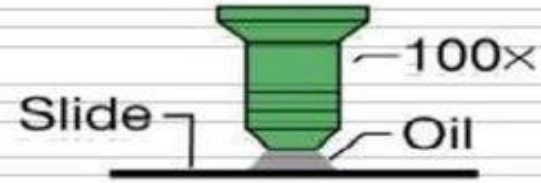


Dry in air



Pass slide through flame to fix

Flood slide with stain; rinse and dry

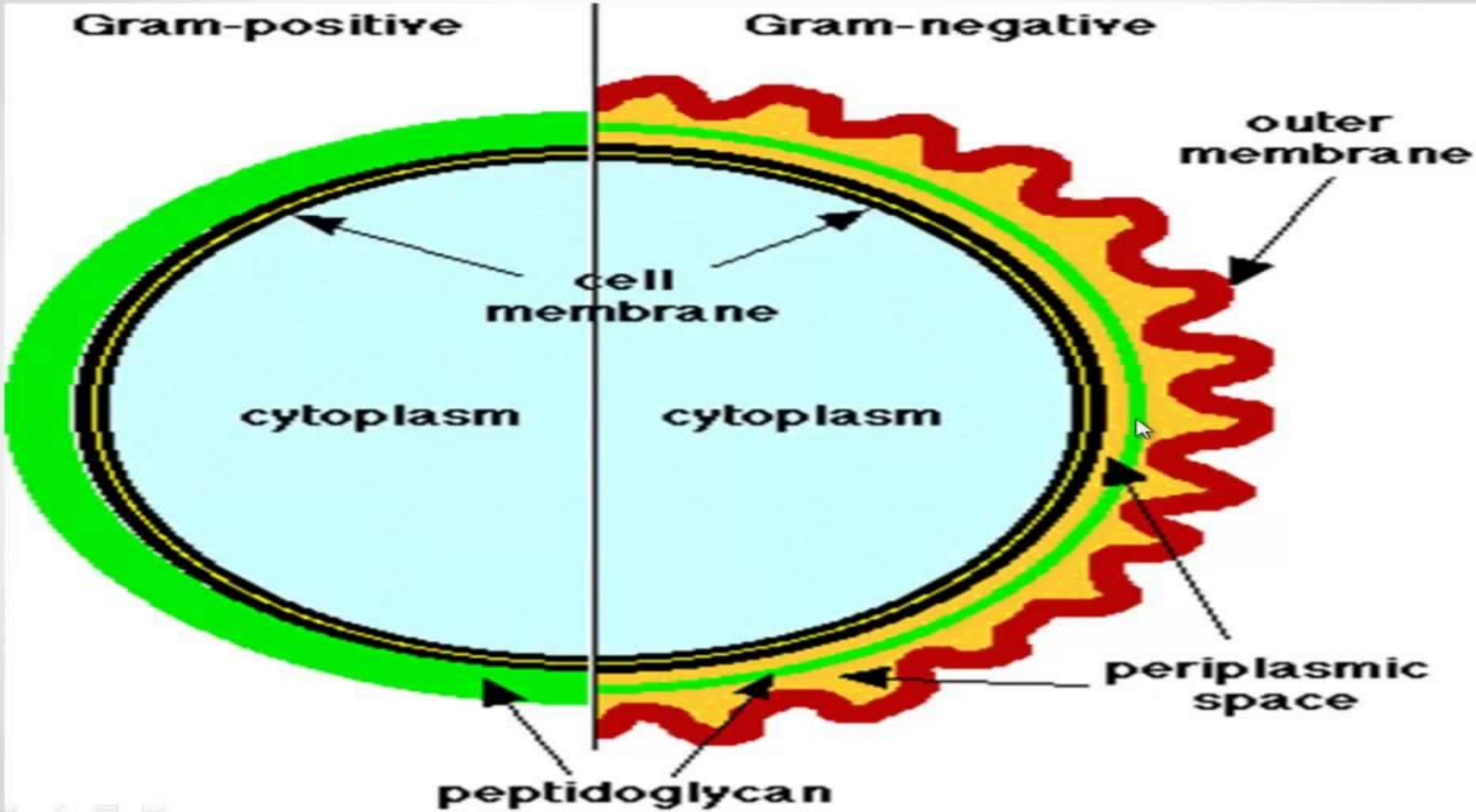



Place drop of oil on slide; examine with 100× objective

# Gram stain

**Gram stain** or **Gram staining**, also called **Gram's method**, is a method of staining used to distinguish and classify bacterial species into two large groups (Gram-positive and Gram-negative).

- Gram staining differentiates bacteria by the chemical and physical properties of their cell walls.
- Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet.
- Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out. They are stained pink by the counterstain, commonly safranin or fuchsine.

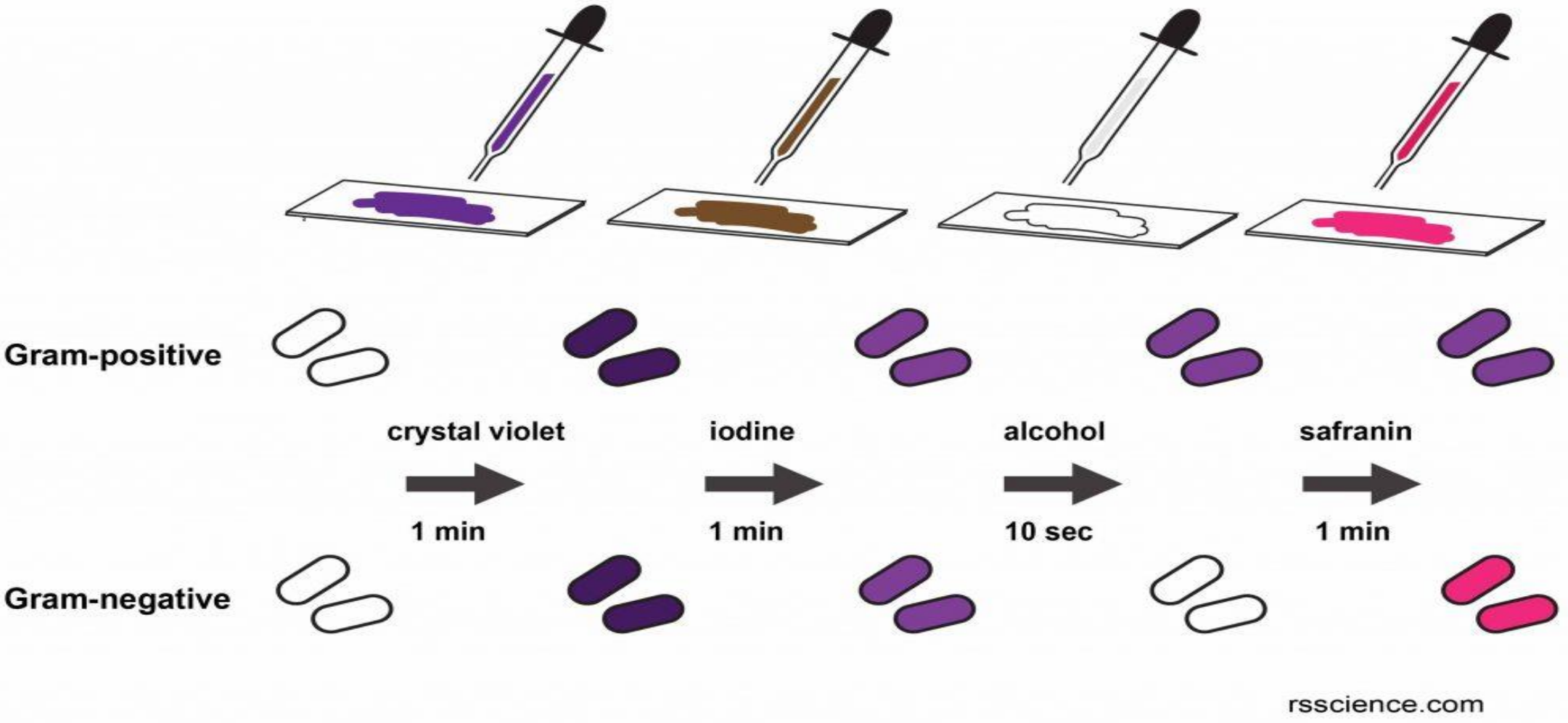

Gram-positive
Gram-negative
outer membrane
cell membrane
cytoplasm
cytoplasm
periplasmic space
peptidoglycan

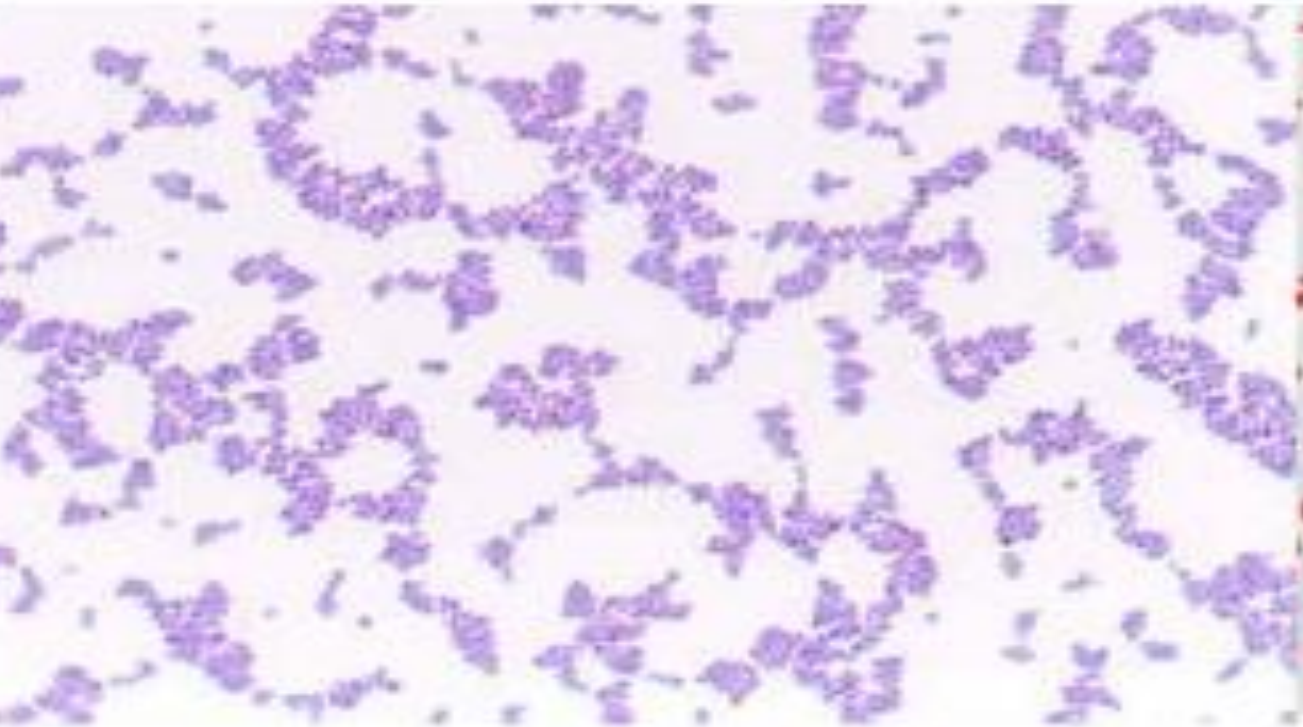
# PROCEDURE

- Step 1- Crystal violet (primary stain) for 1 minute. Water rinse.
- Step 2- Iodine (mordant) for 1 minute. Water rinse.
- Step 3 – Alcohol (decolorizer) for 10-30 seconds. Water rinse.
- Step 4 - Safranin (counterstain) for 30-60 seconds. Water rinse. Blot dry.

- Cells stain purple.
- Cells remain purple.
- Gram-positive cells remain purple. Gram negative cells become colorless.
- Gram positive cells remain purple. Gram-negative cells appear red.

# Gram staining procedure





Gram +ve Bacteria

Gram -ve Bacteria

# THANK YOU…