

Bacterial Staining

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Types of Staining

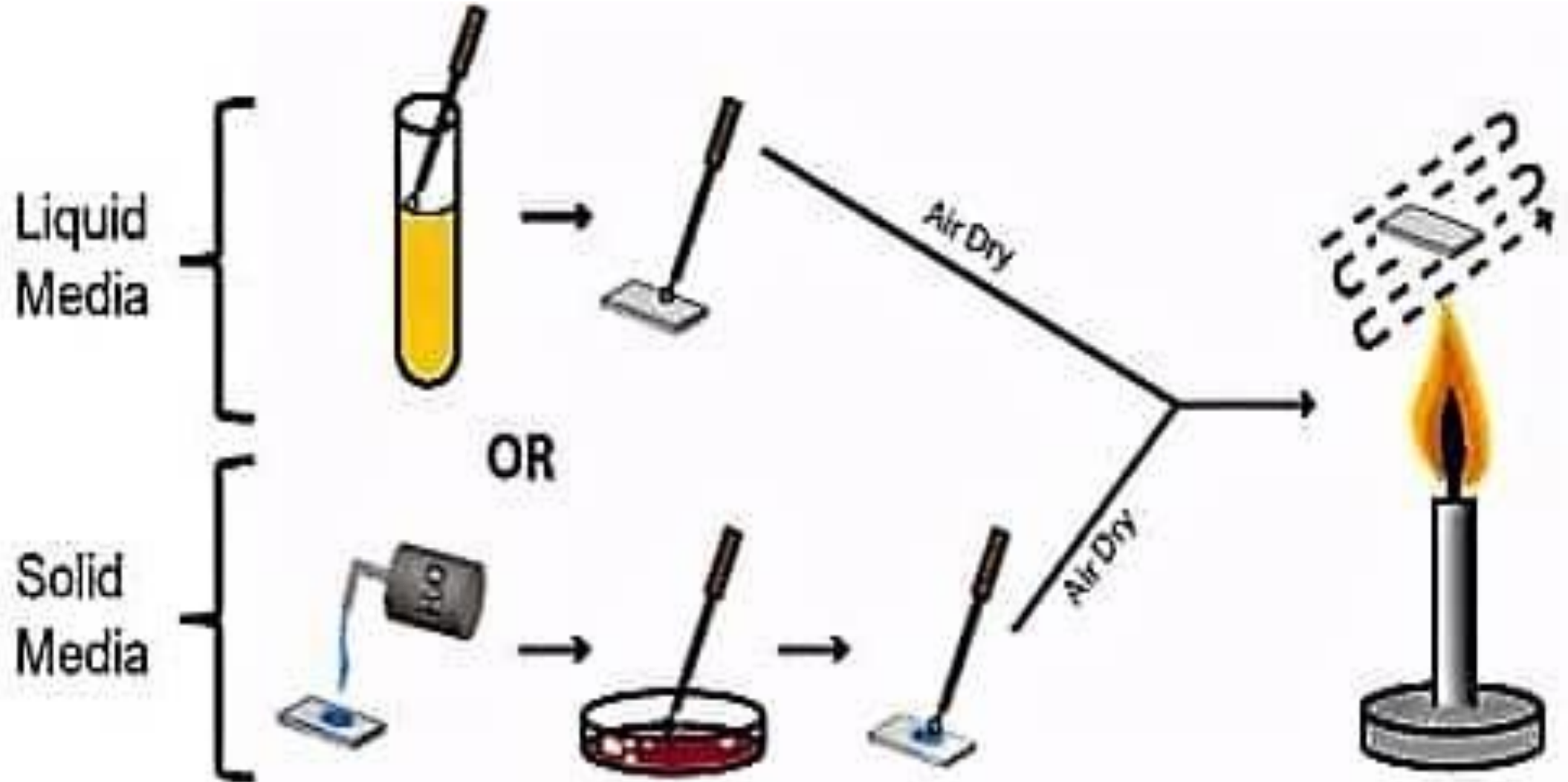
- Simple staining
- Differential staining
- Negative staining
- Impregnation method
- Special staining - for certain bacteria, bacterial spores, parasites & fungi

Preparation of Bacterial Smear

Steps of microbial smear preparation :

1. Handle a clean slide by its edge, label the target place at the bottom side of the slide by drawing a circle with a diameter about 2 cm using a marker .
2. Sterile the loop until reaching the red heat .
3. If the bacterial culture was broth, shake the culture and transfer loopful of broth to the center of the slide and spread over the target circle . While if the bacteria were grown on solid medium , place loopful of water on the slide then transfer inoculums to the water and homogenize the smear.
4. Sterile the loop.
5. Leave the smear to dry at room temperature (by air) .
6. After drying , Pass the slide over the flame to fix the smear (avoid prolonged heating of the slide) .

Preparation of Bacterial Smear



Importance of fixing the smears “Fixation accomplishes three things:

- (1) it kills the organisms;
- (2) it causes the organisms to adhere to the slide; and
- (3) it alters the organisms so that they more readily accept stains (dyes).

Simple Staining

Principle, Procedure and Result Interpretation

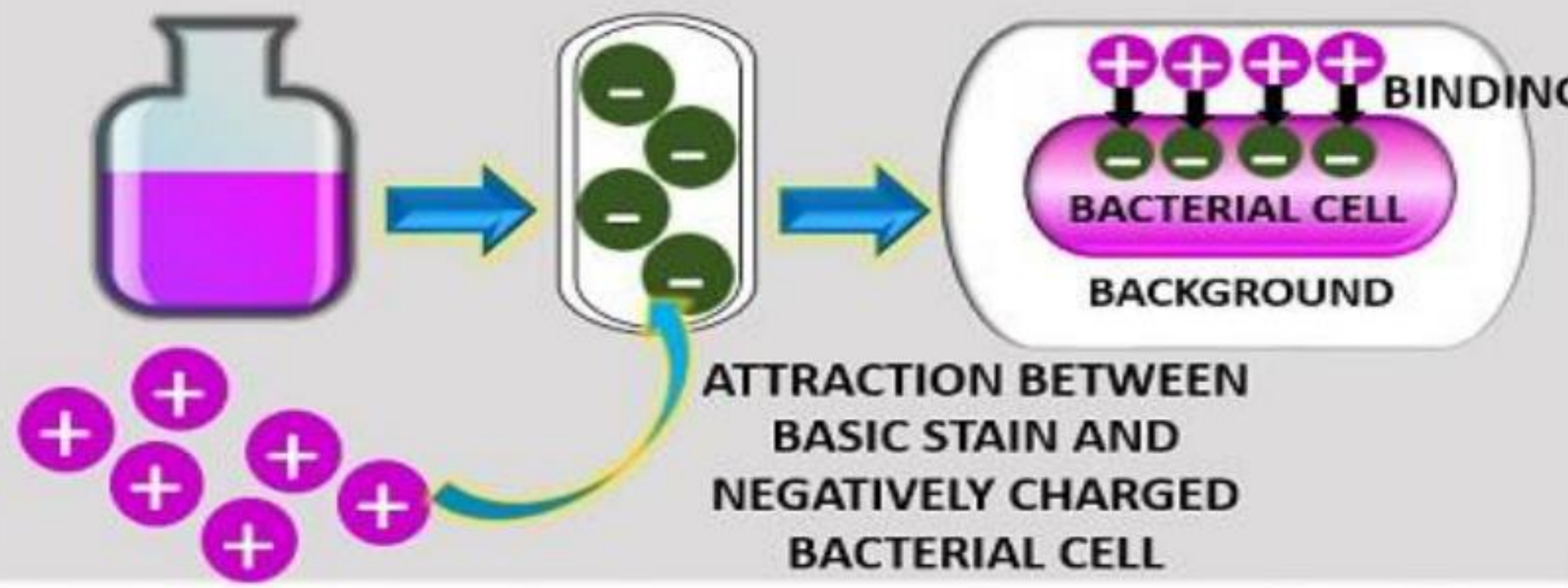
The microorganisms are invisible to the naked eye, and to make them visible, staining is performed that gives divergence to a microscopic image.

Simple staining is one of the conventional staining techniques. From the name, it is quite clear that it is a very simple or direct staining method that uses a single stain only **like methylene blue, safranin, crystal violet, malachite green** etc. called “simple or direct stains”.

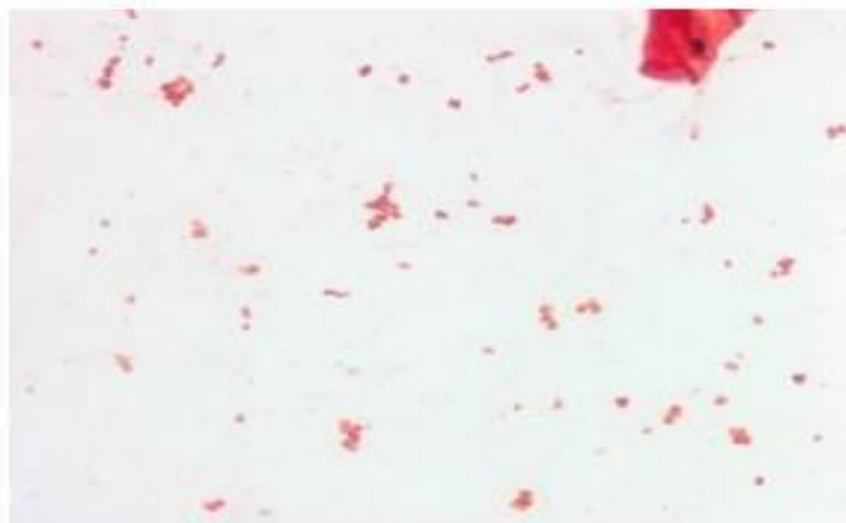
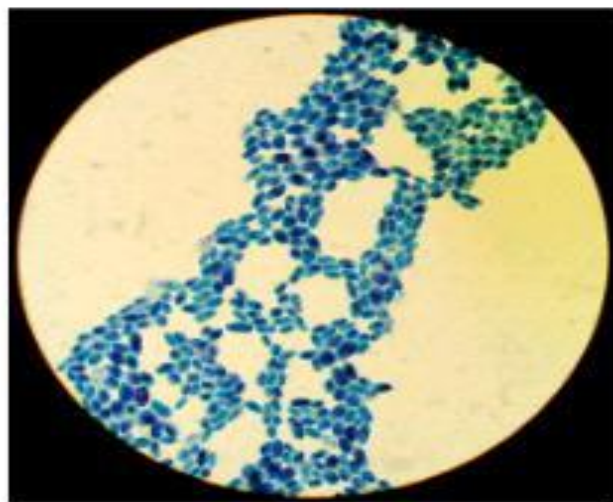
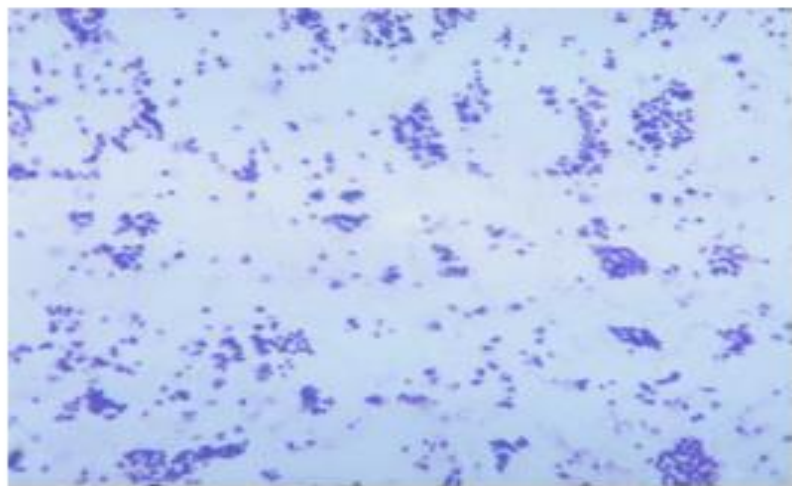
The simple stain can be used as a quick and easy way to determine the **cell shape, size, and arrangement** of bacteria.

**BASIC STAIN
WITH POSITIVE
CHROMOPHORE**

**NEGATIVELY
CHARGED
BACTERIAL CELL**



PRINCIPLE OF SIMPLE STAINING

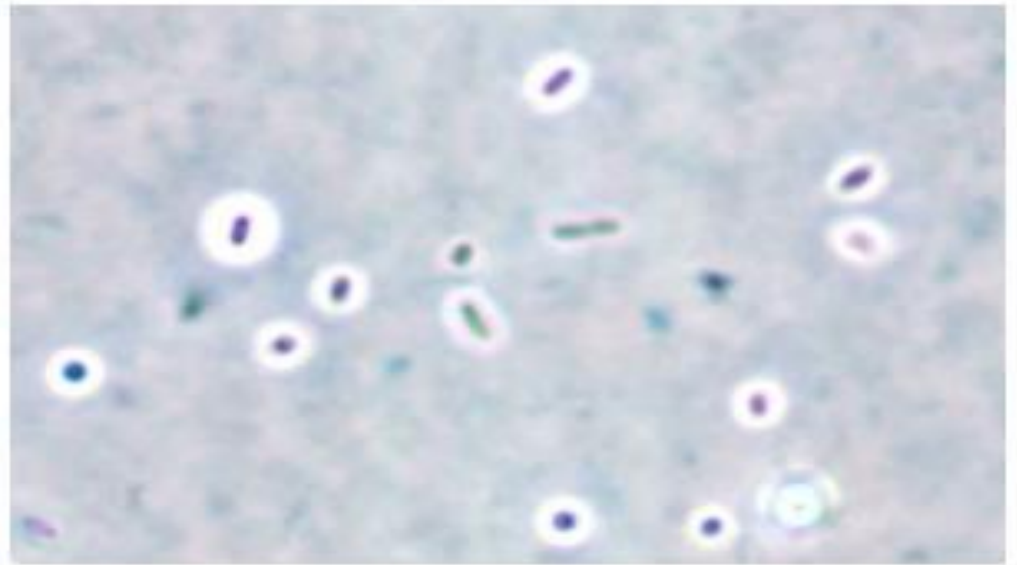


Types of simple staining:

1. Direct / Positive staining : stain Bacterial cell
2. Indirect / Negative staining: stain background of Slide

NEGATIVE STAINS

- Produce colored background against which bacteria stand out in contrast.
- Used for bacterial capsule, spirochetes eg India ink, Nigrosin.







DIFFERENTIAL STAINS

- Imparts different color to different bacteria.
- Most widely used differential stains are:
 - Gram stain &
 - Ziehl Neelsen stain

Gram staining

Named after Hans Christian Gram, differentiates between Gram-positive purple and Gram-negative pink stains and is used to identify certain pathogens.



Principle

- The Gram stain is the most useful and widely employed differential stain in bacteriology.
- It divides bacteria into two groups (Gram positive and Gram negative bacteria).
- The primary stain is crystal violet. It is followed by treatment with an iodine solution, which functions as a mordant, that is, it increases the interaction between the bacterial cell and the dye.

Gram Stain

Principle of staining technique:

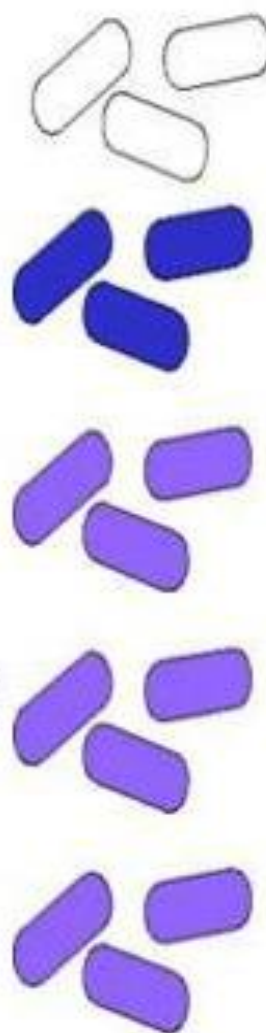
Primary stain:- Crystal Violet

Mordant(fixes the dye):- Iodine

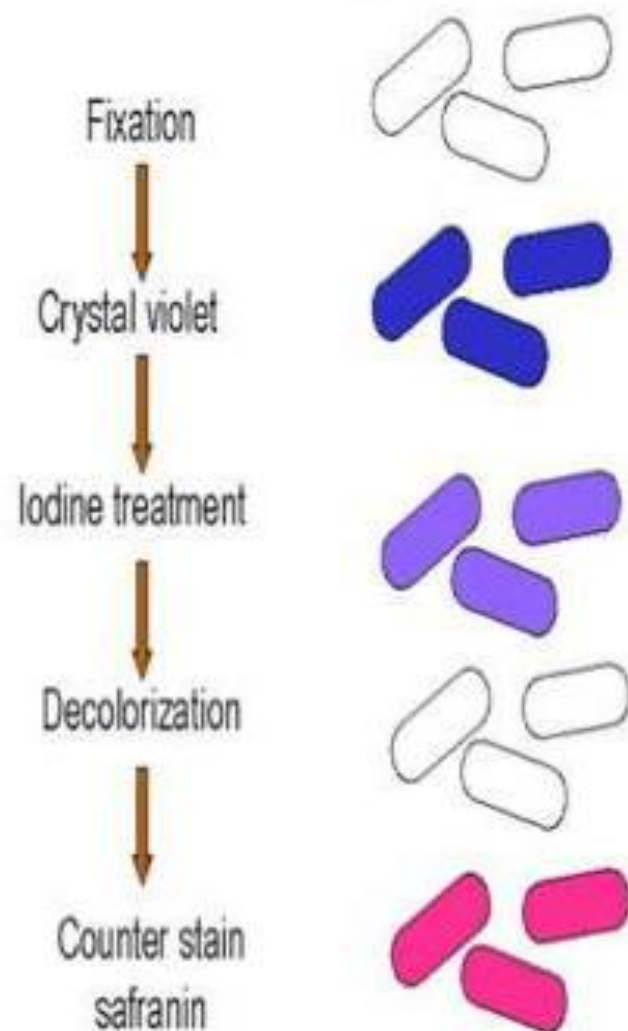
Decolorizing agent:- Alcohol/Acetone

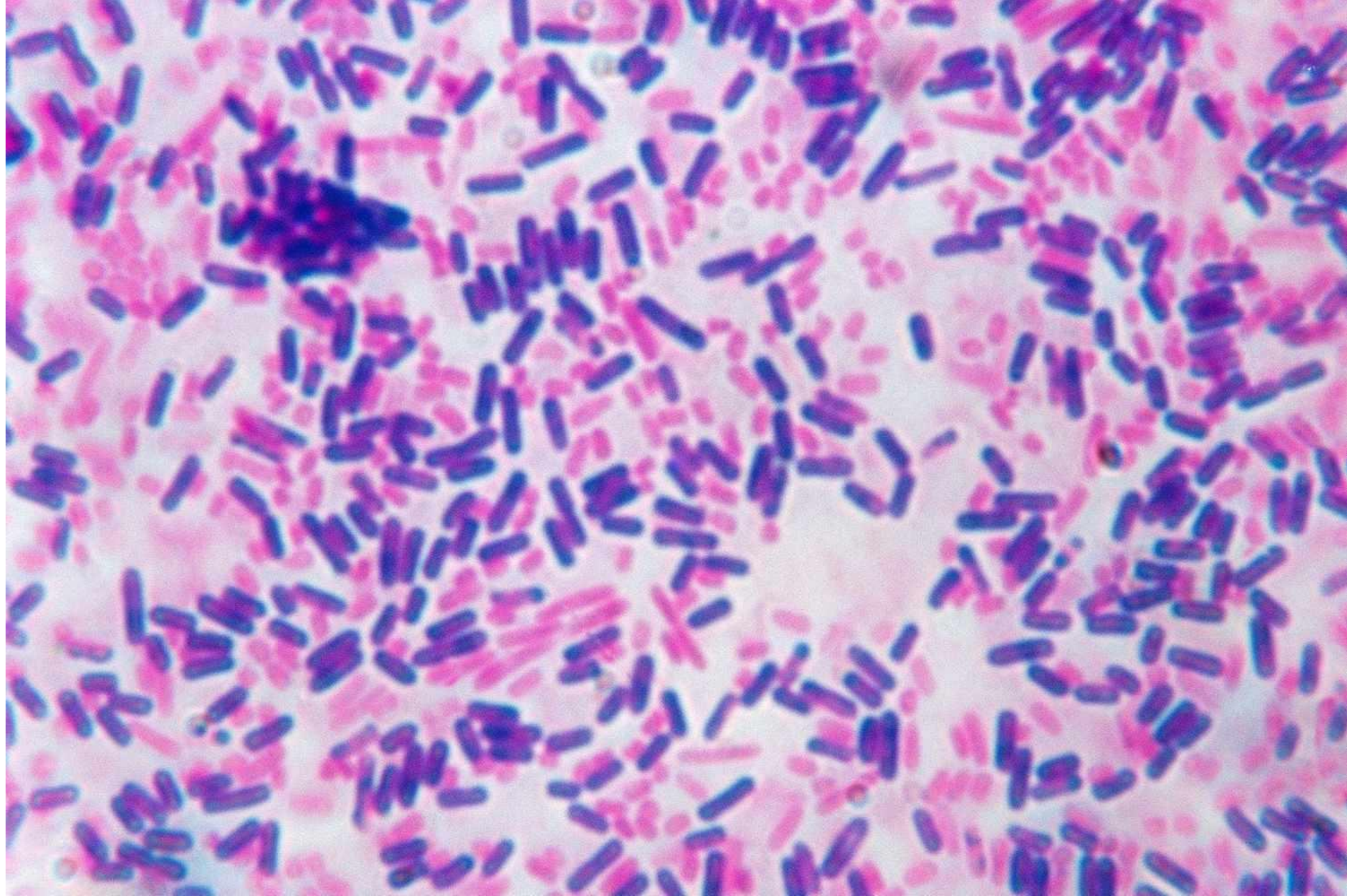
Counter stain;- Safranin

Gram Positive



Gram Negative







Some Microscope Center, Inc.
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Ann Arbor, MI 48106
Tel: 734-994-5990
Fax: 734-994-9090
email: sales@microscope.com

Gram Stain Kit Set of 4

Instructions & Safety Data Sheets
Part # GG0221-KIT



Thank you