

# Gram staining

One of the basic stains in microbiology, **gram staining** gave rise to the division of bacteria into **Gram positive (G+)** and **Gram negative (G-)**. This division is based on the different structure of the bacterial wall.

## Procedure

The sample or bacterial culture to be examined is applied to the slide and the staining solutions are gradually applied. Allow each solution to work for approximately 1 minute<sup>[1]</sup>. The procedure is easily remembered by the abbreviation **VLAS (VLAK)**:

- Crystal **Violet**
- Lugol's solution
- **Alcohol**
- rinse with water
- **Safranin** or **Carbolfuchsin**

## Positivity and negativity

### G+

Gram-positive bacteria have a wall composed of peptidoglycan and polysaccharides through which teichoic acid passes. During staining, the crystalline **violet** enters the cells and forms a **blue** complex color with Lugol's solution. The alcohol is unable to penetrate the cell wall and dissolve the complex. Safranin staining gives the bacteria a deep purple color.

- G+ cocci: *Staphylococcus*, *Streptococcus*, *Enterococcus*;
- G+ bacilli: *Corynebacterium*, *Clostridium*, *Listeria*, *Bacillus*.

### G-

Gram-negative bacteria have a wall consisting of a thin layer of peptidoglycan and a layer of lipopolysaccharide. In the same process, the third step involves **washing out** of the complex with alcohol and **decolorization**. Safranin stains the bacteria red.

- G- cocci: *Neisseria*;
- G- coccobacilli: *Haemophilus influenzae*, *Bordetella pertussis*, *Legionella*, *Brucella*, etc.
- G- bacilli: *Klebsiella*, *E. coli*, *Enterobacter*, *Citrobacter*, *Serratia*, *Vibrio*, *Pseudomonas*, *Proteus*, *Helicobacter pylori*, *Yersinia*, *Campylobacter*, *Salmonella*, *Bacillus fragilis*, etc.

## G labile and non-staining

Some bacteria, especially after long cultivation and multiple passaging, or if they survive the attack of antibiotics against the cell wall (L-forms), can change from G+ to G-.

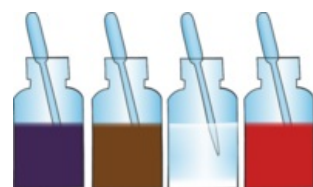
Bacteria that contain a lot of fatty acids and waxes in their wall (*Mycobacterium tuberculosis*) can not be stained by Gram at all.

## Links

### Related articles

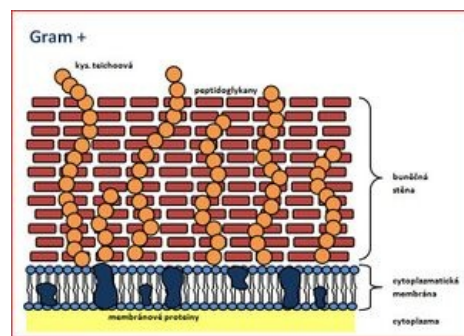
- Burri staining
- Staining by Giemsa
- Hematoxylin-Eosin staining
- Chromosome staining
- Bacteria

### Source

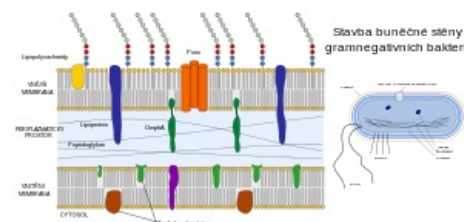


Krystalová violet, Lugolův roztok, Alkohol, Karbolfuchsin

Gram coloring



Gram positive



Gram negative

- RYŠKOVÁ, Olga. *Návody k praktickým cvičením z lékařské mikrobiologie*. 1. edition. Praha : Karolinum, 1997. ISBN 80-7184-307-5.

## Reference

1. JULÁK, Jaroslav. *Praktická cvičení a semináře z lékařské mikrobiologie*. 2. edition. Praha : Karolinum, 2009. 113 pp. ISBN 978-80-246-1141-9.

## Source

- ws:Gramovo barvení