

# Histological evidence of microorganisms

## GRAMM-TWORTH METHOD

Staining makes it possible to divide bacteria into gram-positive (G+) and gram-negative (G-) and, together with morphology (cocci, rods, etc.), enables their basic identification. G+ and G- bacteria differ in the structure of the cell wall and its permeability to dyes.

- Both G+ and G- stain with crystal violet, which forms a blue complex with iodine solution. In the next step, acetone is applied.
- The wall of G+ bacteria remains stained after the application of acetone, while the wall of G- bacteria becomes discolored. This is followed by red/fast green staining (a mixture of neutral red and fast green in ethanol), which stains the wall of G-bacteria.
- The result of the staining is therefore blue G+ bacteria, red G- bacteria, cell nuclei of the surrounding tissues are red, collagen, erythrocytes and cell cytoplasm are stained with different shades of green.

## GIEMS STAIN

Enables morphological identification of protozoa and bacteria.

- A neutral dye is used, created by mixing a basic dye (eg azure B, methylene blue) with an acidic dye (eg eosin).
- Microorganisms, fungi, parasites are blue-violet, nuclei are blue, erythrocytes are pink, cytoplasm is light blue, collagen, muscles, bones are light pink.

## ACID-RESISTANT DYEING

Mycobacteria ( *Mycobacterium tuberculosis*, *M. leprae*) cannot be demonstrated by Gram stain. Their cell wall contains a large amount of waxy substances (long chains of mycolic acid) causing hydrophobicity. Mycobacteria are acid-resistant, i.e. they are able to hold the dye once taken and thus resist discoloration.

- Concentrated carbolfuchsin applied while hot is used for staining. This process will stain any bacteria present. However, it is not possible to decolorize acid-resistant bacteria with acid alcohol.
- Malachite green is used to stain non-acid-resistant bacteria and other structures. Acid-resistant bacteria are red, other structures are light blue to light green.
- the **Ziehl-Neelson test is used to test for *Mycobacterium tuberculosis*** (the causative agent of tuberculosis) . the **Fita certificate** is used to identify *Mycobacterium leprae*.

## WARTHIN-STARRY METHOD (SILVER STAINING)

The Warthin-Starry method is used to detect spirochetes (genera *Borrelia*, *Leptospira*, *Treponema*), *Legionella*, *Bartonella* and *Helicobacter pylori*.

- A solution of silver nitrate is used for staining. The bacterial cell wall is capable of binding silver ions, but their reduction does not occur. The reduction of silver ions to metallic silver is carried out with hydroquinone.
- The bacteria turn dark brown, the background of the section is yellow.

## RESPONSE PASS

The PAS reaction can also be used to detect fungi and amoebae, because the cell wall of fungi contains a large amount of polysaccharides.

- The cytoplasm of amoebae contains glycogen. The principle of dyeing is the oxidation of polysaccharides to aldehydes by periodic acid. Aldehydes subsequently react with Schiff's reagent to produce a pink color.

## Links

## References

- ČÍŽKOVÁ, Kateřina. *Basics of histochemical methods*. - edition. 2018. 50 pp. ISBN 9788024453163.