

Principles of conventional histochemistry in light microscopy

Histochemistry

Histochemical reaction is used to detect inorganic and organic compounds in tissues.

Detection of inorganic compounds

Fe

Fresh tissue can be fixed with 10% neutral formalin or anhydrous ethanol. We do not use fluids containing acids or potassium dichromate for fixation. The tissue is embedded in paraffin or cut on a freezing microtome.

We demonstrate the presence of Fe^{3+} , Fe^{2+} ions in the tissue. To detect Fe^{3+} , we use Berlin (Prussian) blue, the so-called Perls reaction, with which we prove iron, for example, in hemosiderin. Fe^{2+} we use reactions with Turnbullblue (blue coloration)

Ca

Pathological calcium deposits can occur in any area of the body. Calcium can occur in the body in soluble or insoluble form, this must be taken into account, for example, during fixation.

The calcium detection method is most often used to detect insoluble phosphates and carbonates. Substitution methods with silver nitrate are best suited for the routine detection of calcium salts. These methods are based on the reduction of silver, they are mainly methods:

Evidence of calcium according to Kossa
Gohs reaction for the detection of calcium

Detection of organic compounds

Lipids

Tissue in which lipids are to be demonstrated must not come into contact with organic solvents (e.g., high percentage ethanol, xylene, ether, acetone). Consequently, the tissue cannot be embedded in paraffin or in celloidin. However, gelatin embedding or the frozen section method can be used.

Lipids are stained with fat-soluble dyes. These are, for example, sudan red, oil red, octahedral oxide.

Saccharides

Water-soluble sugars such as simple sugars (glucose,...) cannot be histochemically detected. Lowly soluble compounds, polysaccharides or sugar components of proteins or fats are demonstrated.

Sugars are demonstrated by aldehyde groups formed by cleavage of the pyran or furan ring. The aldehyde groups then react with Schiff's reagent to give a red-purple colour (PAS reaction).

Substances that can be observed in this way include

- **Polysaccharides** - hyaluronic acid, chondroitin sulfate, heparin, keratan sulfate, dermatan sulfate
- **Proteoglycans** - perlecan, versican, syndecan
- **Glycoproteins** - thyroglobulin, fibronectin, laminin, collagen, mucins
- **Glycolipids** - cerebrosides, sphingomyelin, lipofuscin

Pigments

Gmelin reaction

DNA

Nucleic acids are basophilic. The Feulgen reaction, which uses Schiff's reagent, is the most suitable for the exact determination of DNA. The Feulgen reaction is used in pathology to diagnose tumors to determine cell ploidy.

Links

Related articles

- Histochemistry
- Hematoxylin-eosin staining
- Staining in light microscopy

Literature

- VACEK, Zdeněk. *Histology and histological technique. Volume 2, Histological technique*. 1. edition. Brno : Institute for Further Education of Health Care Workers, 1996. ISBN 80-7013-202-7.
- MAŇÁKOVÁ, Eva – SEICHERTOVÁ, Alexandra. *Methods in Histology*. 1. edition. Prague : Karolinum, 2002. ISBN 80-246-0230-X.