Basic and special staining methods, principles and results

Staining:

Tissues lack inherent coloration, necessitating staining procedures. These methods enhance the visibility of various tissue components and facilitate their differentiation.

The majority of dyes exhibit properties akin to acidic or basic compounds, establishing electrostatic interactions with ionizable radicals present in tissues. Tissue components bearing a net negative charge (anionic) readily bind with basic dyes (cationic, possessing a positive charge), termed as Basophilic. Conversely, cationic components, such as proteins with numerous ionized amino groups, exhibit an affinity for acidic dyes (anionic, with a negative charge) and are referred to as Acidophilic (also known as eosinophilic or oxyphilic). Neutrophilic describes tissue structures that stain with both basic and acidic dyes.

Examples of basic dyes are:

• Hematoxylin

• Toluidine blue - RNA

• Alcian blue - special dye for staining acid mucopolysaccharides

• Methylene Blue - RNA

Nucelus, Ribosomes, RER

Examples of Acidic dyes:

• Orange G

• Eosin

• Acid fuchsin

• Aniline Blue

Stain the acidophilic components of tissues such as mitochondria, cytoplasm with many mitochondria, lysosomes, secretory granules and collagen.

• Alum hematoxylins / alum mordant / Harris or Mayer´s hematoxylin .

• Iron hematoxylins / iron mordant / Weigert ´s or Heidenhain´s hematoxylin.

Among all dyes, the straightforward pairing of Hematoxylin (a basic dye that stains basophilic components with a negative charge) and Eosin (an acidic dye that stains acidophilic components with a positive charge) is notable. This combination, known as H&E, is widely utilized. Hematoxylin imparts a blue coloration to DNA within cell nuclei and other acidic structures (such as RNA-rich areas of the cytoplasm and cartilage matrix). Conversely, Eosin imparts a pink hue to other cytoplasmic components and collagen. Consequently, this combination effectively highlights the main tissue structures through color contrast.

For visualizing a peripheral blood smear, Wright's stain is recommended. However, Hematoxylin and Eosin stain (H&E) is the most frequently employed stain for routine histological examination of tissues. Lipids are best visualized using a Sudan stain. Silver impregnation techniques, like reticular staining, can be employed to visualize reticular fibers. Resorcin fuchsin and orcein stains are optimal for displaying elastic fibers in elastic cartilage.

Staining:

1. Progressive – dye is taken up by the tissue and it is not removed, Regressive – Overstaining and then differentiation of excess dye.

2. Successive – Staining in successive baths of different dyes, Simultaneous – dyes dissolved in one bath.

3. Vital Stain – stain that can be applied on living cells without killing them. Supra Vital staining – living cells that have been removed from an organism Intra Vital staining – Injecting or otherwise introducing the stain into the body.

4. Direct vs with mordant (aluminum, tungsten or ferric salts) – mordant enhance staining ability of some dyes, e.g. alum or iron haematoxylins.

Staining procedure:

1. Deparaffinization: Xylene (5 min) - Xylene (5min) - EtOH 100% (ethanol) (3-5min) - EtOH 96% (ethanol) (3- 5min) - Washing in water (5min)

2. Staining: Alum Hematoxylin (3-10min) - Rinse in water - differentiation in acid ethanol - washing in tap water - Eosin (0.5%) (1-3min) - Rinse in water - Differentiation excess dye in 80% ethanol.

3. Dehydrating : Ethanol 96% (1-3min) - Ethanol 100% (3-5 min) - Carbo-xylene (3-5min)

4. Clearing: Xylene (5min) * 2

5. Mounting – coverslipping. Once any staining technique has been completed, the cover slip must be mounted on the section. Mounting media are desired to have high refractive index (1.53-1.54) and a good preservative capability. Mounting in Canada Balsam or synthetic\water insoluble resin.

Results of staining:

• Nuclei – blue \ purple

• Cytoplasm – varying shades of pink

• Collagen fibers – pink or red

• Muscles – deep pink or red

• RBC – reddish pink or orange

Other fundamental staining techniques include Masson's trichrome, Azan, and Weigert van Gieson stains. These methods are particularly useful for selectively highlighting connective tissue components, specifically collagen fibers. Collagen fibers are known to stain yellow with Saffron, blue with Aniline blue, green with light green, and red with acid fuchsin.

Special Staining methods:

Based on:

1. Specific affinity of dyes to a specific tissue component (e.g. staining of elastic fibers by orcein or aldehyde fuchsin)

2. Solubility of dyes in certain substances (e.g. staining lipids by Sudan dyes)

3. Reactive groups of carbohydrates reducing metallic silver from the alacline silver solution.

• Staining of elastic fibres and membranes (elastin): orcein – reddish brown, aldehyde fuchsin – violet, resorcin-fuchsin – black ethanolic solution necessary because hydrophobia of elastin

• Cytological staining – Heidenhain´s iron hematoxylin – demonstrates electively cytological details – nuclei (chromatin), mitochondria, centrioles, secretory granules, myofibrils, etc.

• In addition to tissue staining with dyes, metal impregnation techniques usually using silver salts are common method of visualizing certain ECM fibers and specific cellular elements in nervous tissue. Impregnating methods – demonstration of reticular fibers (Gomori), based on ability to reduce metal silver from its unstable salts (e.g. amoniacal Ag solution) – brown – brown-black precipitate

References

- JUNQUIERA, Anthony – MESCHER,. *Junqueira's Basic Histology.* 16. edition. McGraw Hill LLC, 2001. 576 pp. ISBN 1260462978.