

Histopathological Diagnosis

PREPARATION OF HISTOLOGICAL SPECIMENS



PERIODIC ACID-SCHIFF REACTION (PAS)

- The Schiff reagent is a bleached basic fuchsin that reacts with aldehyde groups. This reaction results in a **deep red colour** in the section. It is the basis of the PAS stain.
- PAS stains **carbohydrates and carbohydrate rich macromolecules** a **deep red colour (magenta)**.



PERIODIC ACID-SCHIFF REACTION (PAS)

- **PAS will therefore stain up:**
- glycogen the intracellular storage form of carbohydrate in cells
- Mucus in cells and tissues, Basement membranes, and Brush borders of kidney tubules and small and large intestines
Reticular fibres (i.e. collagen) in connective tissue and
Cartilage.
- In the example shown above, The mucin produced by goblet cells is stained a purple colour by this stain.



MASSON'S TRICHROME

- This is often used to stain connective tissue.
- Tri-chrome - means the technique produces three colours.
- Nuclei and other basophilic (basic-liking) structures are stained blue, cytoplasm, muscle, erythrocytes and keratin are stained bright-red.
- Collagen is stained green or blue, depending on which variant of the technique is used.



ALCIAN BLUE

- Alcian blue is a mucin stain that stains certain types of mucin blue.
- Cartilage is also stained blue. It can be used with H&E, and with van Gieson stains.



VAN GIESON

- This stains collagen red, nuclei blue, and erythrocytes and cytoplasm yellow.
- It can be combined with an elastic stain that stains elastin blue/black.
- It is often used for blood vessels and skin.



RETICULIN STAIN

- Stains reticulin fibres blue/black. Used with H&E



AZAN

- Nuclei are stained **bright red**, collagen, basement membrane and mucin are stained **blue**, muscle and red blood cells are stained **orange to red**.
- Good for staining connective tissue and epithelium.



TOLUIDINE BLUE

- A basic stain that stains acidic components various shades of blue.
- It is usually used for thin acrylic or epoxy sections.
- MAST CELLSSSS!!!



SILVER AND GOLD METHODS

- Sometimes used to demonstrate fine structures such as cell processes in neurones.
- Produces a black, brown or golden stain.



SUDAN BLACK AND OSMIUM

- These dyes stain lipid-containing structures such as myelin a brownish-black colour.



OIL RED O

- Oil Red O is one of the dyes used for Sudan staining. Similar dyes include Sudan III, Sudan IV, and Sudan Black B. **The staining has to be performed on fresh samples**, as alcohol fixation removes most lipids.
- Oil Red O largely replaced Sudan III and Sudan IV, as it provides much **deeper red color** and the stains are therefore much easier to see.



NISSL AND METHYLENE BLUE

- A basic dye used to stain the rough ER in neurones.



GIEMSA

- Usually used for staining blood and bone-marrow smears.
- Nuclei are stained dark-blue to violet, cytoplasm pale blue, erythrocytes pale pink.



CONGO RED

- Congo red is used for staining in **amyloidosis**, and for the cell walls of plants and fungi, and for the outer membrane of Gram-negative bacteria.



ZIEHL–NEELSEN STAIN

- The Ziehl–Neelsen stain, also known as **the acid-fast stain**.
- It is a special bacteriological stain used to identify acid-fast organisms, mainly **Mycobacteria**. *Mycobacterium tuberculosis* is the most important of this group because it is responsible for tuberculosis (TB).
- Acid-fast organisms like *Mycobacterium* contain large amounts of lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain.



ZIEHL–NEELSEN STAIN

- It can also be used to stain a few other bacteria, such as *Nocardia*. The reagents used for Ziehl–Neelsen staining are – carbol fuchsin, acid alcohol, and methylene blue. Acid-fast bacilli are bright red after staining.
- It is also useful in the identification of some protozoa, namely Cryptosporidium and Isospora.
- Acid-fast bacteria retain carbol fuchsin so they appear **red**.

