HISTOCHEMICAL STAINING

AND TROUBLESHOOTING

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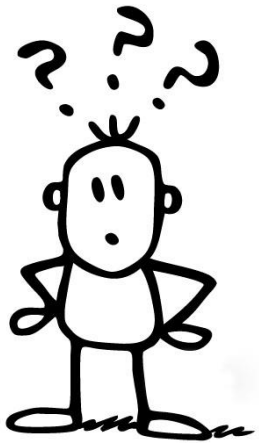
What are "special stains"?

- Alternate staining techniques and histochemical procedures
- Used to visualize particular tissue structures, elements, or even microorganisms not identified by H&E staining.



COMMONLY USED SPECIAL STAINS

- Periodic Acid-Schiff (PAS)
- Enzymatic Digestion techniques (PAS-D)

Periodic Acid-Schiff (PAS)

- **Purpose**:

➢ Used for demonstration of

- Glycogen
- Basement membranes
- fungus
- Neutral mucin

Periodic Acid-Schiff (PAS)

- **Glycogen** is present in liver, kidney, skeletal and cardiac muscle
- **Quality Control**

- Appendix
- Kidney for basement membrane
- Liver with glycogen
- Cervix (include both endocervix and ectocervix)

Periodic Acid-Schiff (PAS)

The presence of glycogen and its distribution patterns are significant in diseases such as:

- Glycogen storage disease of the liver
- Pompe disease- the build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system

Periodic Acid-Schiff (PAS)

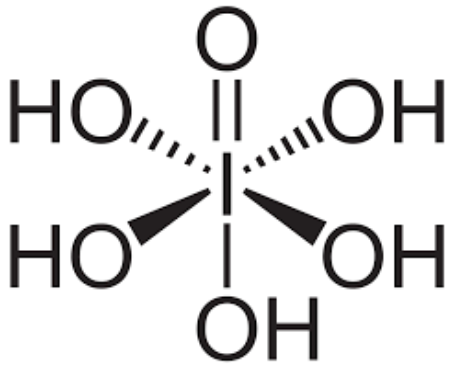
The presence of glycogen and its distribution patterns are significant in diseases such as:

- Rhabdomyosarcoma- a connective tissue cancer
- Mesothelioma

Periodic Acid-Schiff (PAS)

Principal:

- The hydroxyl group (OH) of the carbohydrate molecule is oxidized to aldehyde (CHO) group by **periodic acid**.
- These aldehyde groups react with **Schiff's reagent** to form a magenta-coloured compound



Method

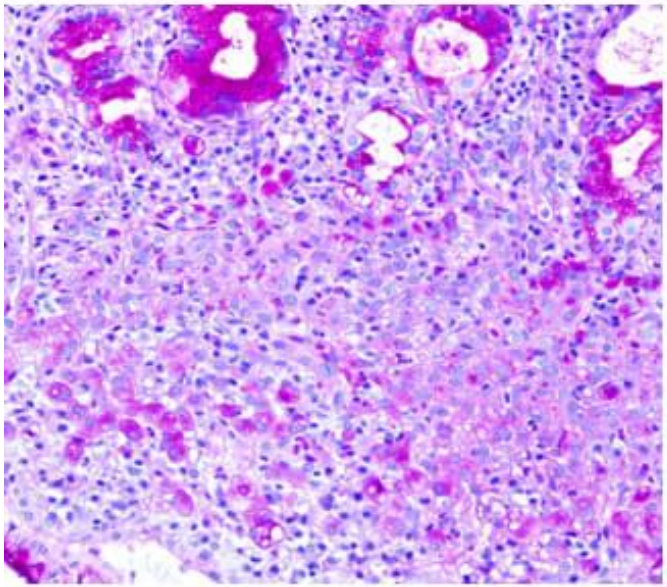
1. Sections to water
2. Oxidize in periodic acid for 10 minutes.
3. Wash well in tap water for 2 minutes.
4. Treat with Schiffs reagent for 15 minutes.
5. Wash well in running water for 10 minutes.
6. Counterstain with heamatoxylin for 30 seconds.
7. Differentiate and “blue” in ammonia water.
8. Dehydrate, clear and mount in DPX.

Result

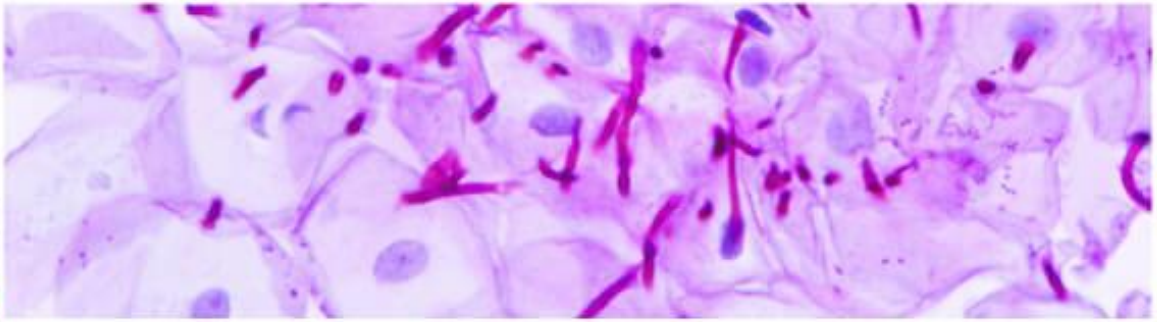
Nuclei	Blue
Glycogen/ fungus	**bright red/purple**
Collagen, mucin, heparin, cartilage and fibrin	**shades of red/pink**

Examples

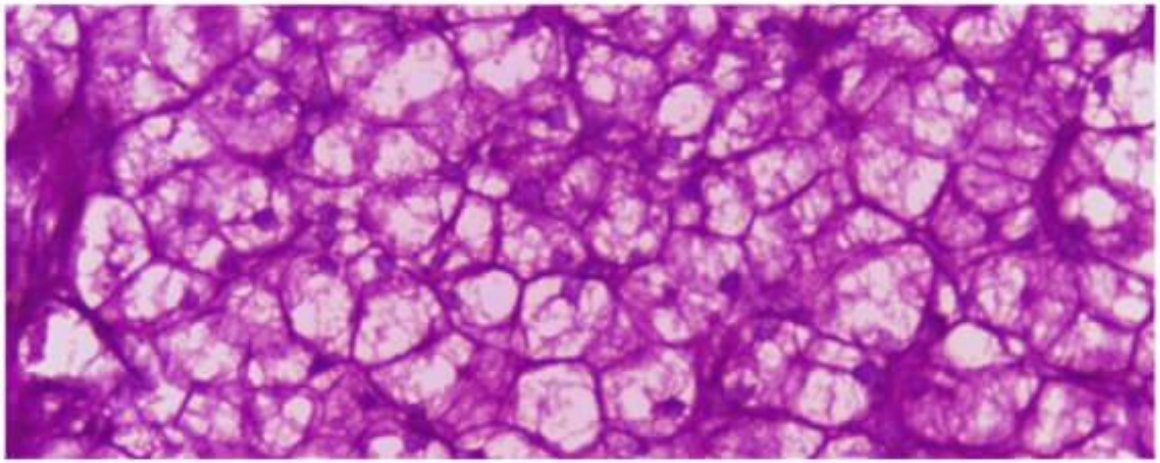
Periodic Acid-Schiff (PAS) Staining



Gastric signet ring cell carcinoma histopathology, PAS stain



Esophageal candidiasis, PAS stain



Liver in glycogen storage disease, PAS stain

Enzymatic digestion techniques

- The amylase or diastase techniques for glycogen digestion are commonly utilized in laboratories to enhance the specificity of the PAS technique.
- The distinction of mucins from glycogen can be problematic when using the PAS technique.

PAS Diastase reaction (PAS-D)

- Glycogen is diastase sensitive, hence section containing glycogen when pretreated with diastase, the enzyme will digest the glycogen and will give negative PAS reaction.
- Typically alpha-amylase such as human saliva may be used in such a situation

PAS Diastase reaction (PAS-D)

PURPOSE:

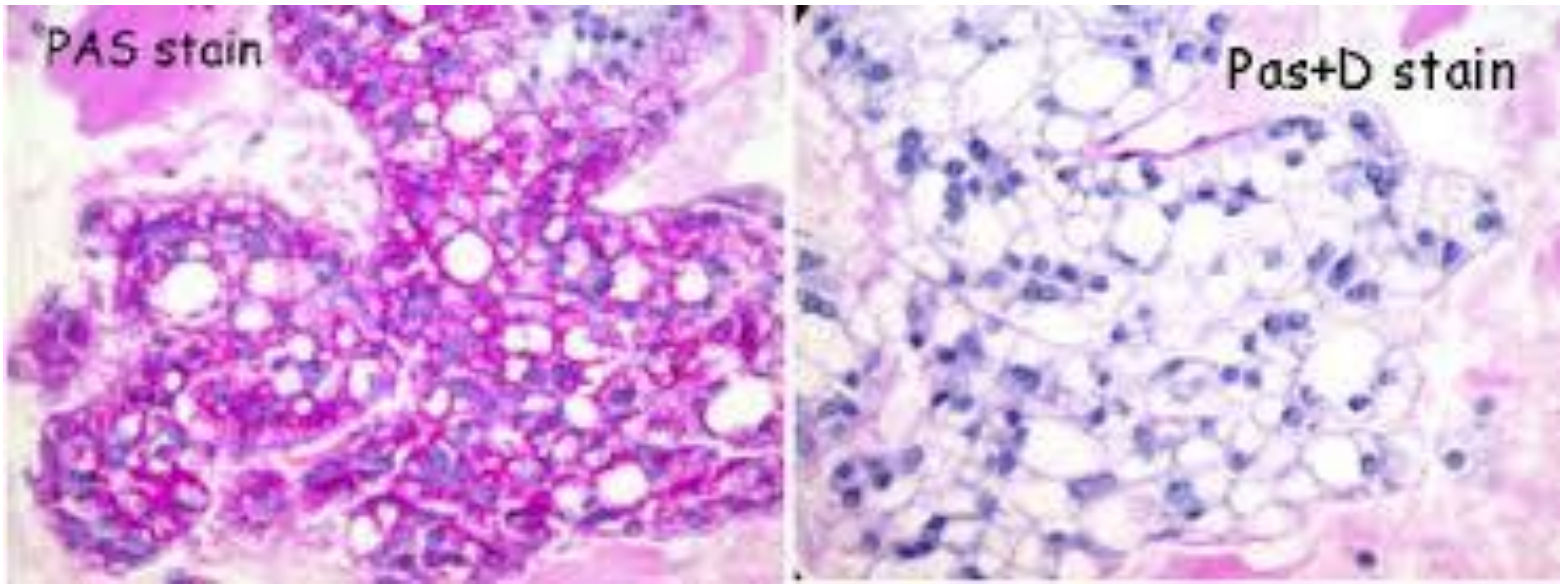
- to differentiate glycogen from other PAS positive elements such as mucin that may be present in the tissue sample.

PRINCIPLE:

- Diastase and α-amylase act on glycogen to depolymerize it into smaller sugar units (maltose and glucose) that are washed out of the section.

Results

- Glycogen staining should be absent in the diastase-treated slide.



Troubleshooting

- Make sure the periodic acid is fresh and that the Schiff's solution is stable.
- Test for Quality of Schiff Reagent

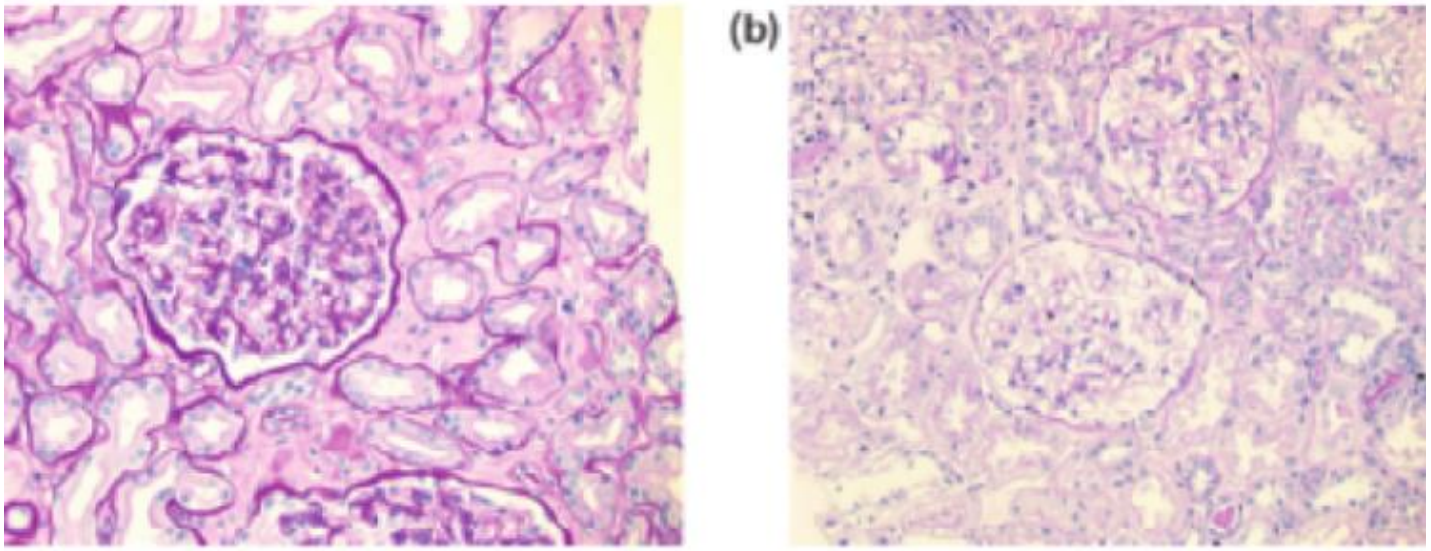
→ Add drops of schiff's reagent to formalin. Active Schiff's reagent will quickly change the colour of formalin to pink. If the reaction is delayed and the resultant colour is a deep blue-purple, the solution is breaking down.

- Unopened Schiff's solution need to store at room temperature in the dark. After the bottle has been opened store well-sealed at 4-8 ° C. Discard the solution if it turns reddish.

Troubleshooting

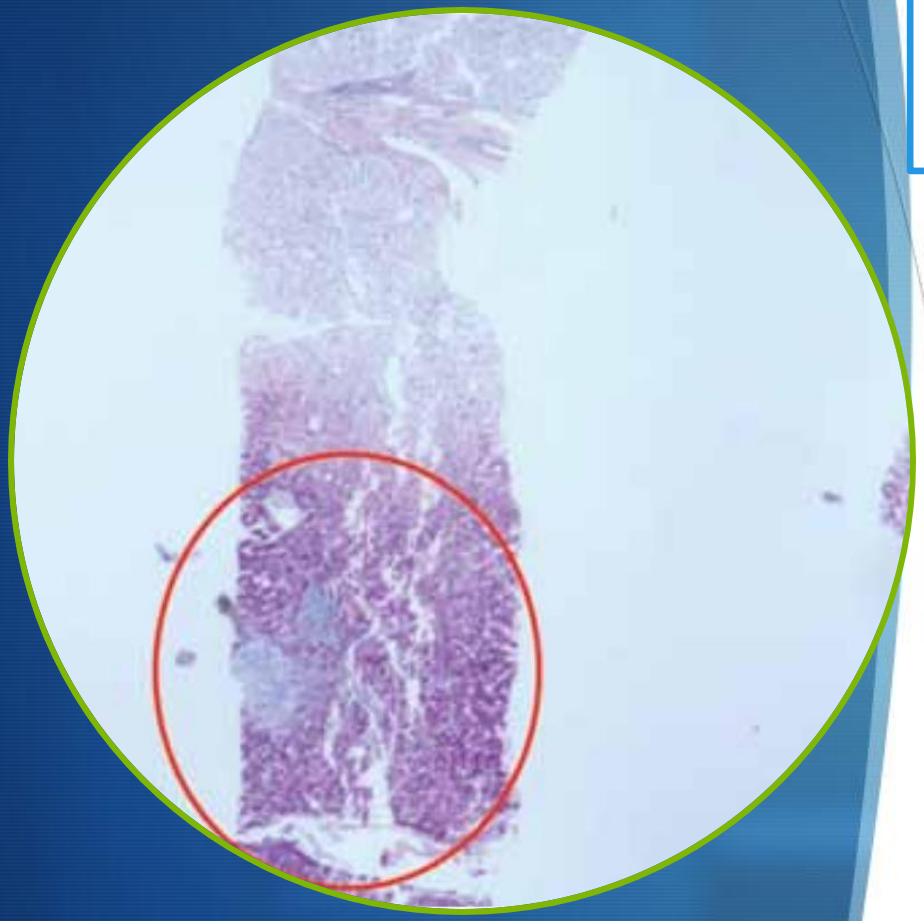
- The intensity of reaction will depend to some extent on the length of treatment with the periodic acid and Schiffs solutions.
- Increasing the oxidation time will usually increase the depth of staining.
- Longer applications of Schiff's reagent also usually increase the depth of staining.

Troubleshooting



PAS staining of basement membranes in the kidney. (a) Good staining. (b) Poor staining

Troubleshooting



Periodic Acid-Schiff Diastase (PAS/D) staining of liver tissue.

The uneven staining is due to amylase not spreading evenly causing part of the liver section to stain undigested glycogen.

References

- Orchard,G. and Nation,B. (2012). *Histopathology*, Oxford University Press, USA
- Carson, F.L. and Hladik, C. (2009). *Histotechnology:A Self-Instructional Text*, Third Edition, American Society for Clinical Pathology Press, USA
- Suvarna, S.K. and Layton, C. *et al.* (2013). *Bancroft's Theory and Practice of Histological Techniques*, Seventh Edition, Churchill Livingstone Elsevier, UK

Thank You

- Thank you for listening to my presentation!
- I hope you enjoyed it!