PERIODIC ACID-SCHIFF (PAS)

Periodic acid
.5% periodic acid in DHOH

Schiff’s reagent
Dissolve 1g. basic fuschsin in 200ml of boiling distilled water, removing the flask of water from heat just before adding the basic fuchsin (do not add while still on hotplate). Allow to cool to 50°C, then add 2g. potassium metabisulphite with mixing. Allow to cool to room temperature then add 2 ml HCL, mix, add 2 g activated charcoal and leave overnight in the dark at room temperature. Filter through a #1 Whatman paper when the solution is either clear or pale yellow. Store in dark container at 4°C.

1. Dewax and bring to DHOH.
2. Treat with periodic acid for 5 minutes.
3. Wash well with several changes of DHOH.
4. Stain with Schiff’s rgent for 15-20 minutes.
5. Wash in running tap water 5-10 minutes.
6. Stain nuclei with hematoxylin(3 dips for Viju’s samples), differentiating with acid alcohol and blueing as usual.
7. Dehydrate to xylene substitute and coverslip.

Results:
glycogen and other periodic-reactive carbohydrates--magenta nuclei--blue
AMYLASE DIGESTION:
1% Amylase in DHOH

After dewaxing and hydrating slides, treat in amylase for 1 hour at 37°C. Rinse 5-10 minutes in running tap water, then continue with PAS stain.

From the internet:
Description: This method is used for detection of glycogen in tissues such as liver, cardiac and skeletal muscle on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The glycogen, mucin, and fungi will be stained purple and the nuclei will be stained blue.

Fixation: 10% formalin.
Section: paraffin sections at 5 um.

Solutions and Reagents:

0.5% Periodic Acid Solution:
Periodic acid ---------------------- 0.5 g
Distilled water -------------------- 100 ml

Schiff Reagent:
Test for Schiff reagent: Pour 10 ml of 37% formalin into a watch glass. To this add a few drops of the Schiff reagent to be tested. A good Schiff reagent will rapidly turn a red-purple color. A deteriorating Schiff reagent will give a delayed reaction and the color produced will be a deep blue-purple.

Mayer’s Hematoxylin Solution:

Procedure:
1. Deparaffinize and hydrate to water.
2. Oxidize in 0.5% periodic acid solution for 5 minutes.
3. Rinse in distilled water.
4. Place in Schiff reagent for 15 minutes (Sections become light pink color during this step).
5. Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color).
6. Counterstain in Mayer’s hematoxylin for 1 minute.
7. Wash in tap water for 5 minutes.
8. Dehydrate and coverslip using a synthetic mounting medium.