Periodic acid - Schiff (PAS) reaction

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The periodic acid-Schiff (PAS) reaction to demonstrate the presence of 1,2-glycol groupings is one of the most widely used histochemical methods. The term "PAS" was introduced by JFA MCMANUS (1946, 1948) who made significant experiments on periodate oxidation techniques.

H BAUER (1933) is credited with the earliest report that the oxidation of sugars (1,2-glycols) with chromic acid created aldehydes which bind the *Schiff* molecule. In the meantime, many studies on the aldehyde-Schiff reaction using various oxidizers have shown that the strength of the oxidizer, the duration of the exposure and the density of sugars in the tissue determine the location where the Schiff molecule will bind.

When tissue sections are treated with periodic acid, glycols are oxidized to aldehydes. After reaction with Schiff's reagent (the main components are pararosaniline and sodium metabisulfite), a pararosaniline adduct is released that stains the glycol containing elements in the tissue section pink to red. A number of cellular elements may be demonstrated with the PAS reaction, e.g. glycogen, basement membranes sulfo- and sialomucins, neutral mucosubstances.

Aprt from our immunohistological stainings, tissue staining with the periodic acid-Schiff method is a routine procedure in our experimental hepatocarcinogenesis models and gives additional informations on the structure-function relationship of differentiating hepatocytes.*

Periodic acid-Schiff method for glycogen (mod. McManus)

Chemicals	**Chemical solution**
Pararosaniline (C.I. 42500) or ready-made Schiff reagent (Merck, Chroma) Schiff's reagent from Merck is prepared in this way: Pararosaniline (C.I. 42500): (a) 0.5g pararosaniline dissolved in 15.0 mL 1 M HCl (b) 0.5 g sodium disulfite dissolved in 85.0 mL distilled water (c) mix both solutions (a) and (b) and allow to stand for 24 hours at room temperature (d) 0.3 g animal charcoal is added and	• Periodic acid solution: 0.8 g periodic acid dissolved in 100.0 mL distilled water • Schiff reagent (ready-made or self-made) according to FEULGEN and ROSSENBECK • sodium disulfite stock solution: 10.0 g sodium disulfite dissolved in 100.0 mL distilled water • sodium disulfite wash solution: 20.0 mL sodium disulfite stock *plus* 20.0 mL 1M HCl *plus* 400 mL distilled water

* Dyes and other chemicals in histological staining can be toxic. They must be handled with care

vigorously shaken for 15 sec (e) the solution is filtered (e.g. Whatman # 2 filter paper (f) solution is stored at 4ºC in a brown glass bottle Periodic acid Sodium disulfite Ethanol 1 M HCl Distilled water	

Staining procedure

Deparaffinated sections (with or without previous immunostaining) are passed through distilled water and stained:

– Periodic acid solution	5-10 min
– distilled water	2 x 5 min
– Schiff reagent	15-20 min
– sodium disulfite wash solution	3 x 2 min
– distilled water	3 x 5 min
– 70% ethanol	2 x 1 min
– 95% ethanol	2 x 1 min

Slides are dehydrated in absolute ethanol, cleared in xylene or xylene substitute and mounted in resinous medium under coverglass

Schiff reagent according to Feulgen and Rossenbeck

Good quality Schiff's reagent can be obtained commercially. The reagent, however, is easily self-made following the formulation of FEULGEN and ROSSENBECK.

Chemicals	**Chemical solution**
Pararosaniline (C.I. 42500) Sodium metabisulfite, 10% in distilled water 1 M HCl Distilled water Activated charcoal (powder)	(a) 200.0 mL distilled water are heated to boil, then take from the heat and add (b) 1.0 g Pararosaniline (be aware, it foams) shake well until the dye is dissolved, cool down to 50°C and add (c) 20 mL 1 M HCL mix well, cool to 25°C and add (d) 10.0 mL 10% sodium metabisulfite mix well, stopper tightly, store in the dark for 1-2 days and add (e) 2.0 g activated charcoal, shake for 1 min and filter the solution (e.g. Whatman # 2 filter paper) (f) solution is stored at 4ºC in a tightly stoppered brown glass bottle

References for further readings

Schiff H (1866)
Feulgen R and Rossenbeck H (1924)
Bauer H (1933)
McManus JFA (1946)
Lillie RD (1947)
McManus JFA (1948)
Hotchkiss RD (1948)
McManus JFA and Cason JE (1950)
Pearse AGE (1980)

Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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