

VAN GIESON'S PICROFUCHSIN

Purpose: Staining connective tissue fibres.

Logistics: turn around time: 5 - 15 minutes (without or with counterstain, resp.)

Specimen: Frozen tissue sections. (times will differ for paraffin sections)

Fixation: Any fixative

Abstract: Collagen and most reticulin stain selectively with acid aniline dyes (aniline blue, acid fuchsin, methyl blue) from fairly strong acid solutions. The acid most used is picric acid, which in addition to providing acidity and acting as a counterstain for muscle and cytoplasm, also appears to form a complex with the dyes mentioned. This complex seems to have a special affinity for collagen.

Stock Reagents:

celestine blue
Mayer's hemalum

iron alum
glycerol

xylol
95 % ethanol
saturated aqueous picric acid

100 % ethanol
acid fuchsin
conc.HCl

Working Solutions:

1. Celestine blue hemalum: Dissolve 2.5 g iron alum overnight at room temperature in 50 ml distilled water; to this add 0.25 g celestine blue and boil for 3 minutes. Filter when cool and 7 ml glycerol.
2. Mayer's Hemalum:
3. 1 % Aqueous acid fuchsin: Dissolve 0.2 g acid fuchsin in 20 ml distilled water.
4. Van Gieson's Picrofuchsin: Add 20 ml of 1 % acid fuchsin to 180 ml saturated aqueous picric acid. Add 0.5 ml concentrated HCl.

Supplies: Forceps, staining dishes, staining racks, filter paper or absorbent paper, permount, coverslips

Procedure:

1. Cut 4- 6 u cryostat sections
2. Let sections dry.
3. (Optional) fix sections.
4. Stain for 5 minutes in celestine blue hemalum.
5. Rinse in tap water and stain for 5 minutes in Mayer's hemalum.
6. Rinse in tap water.
7. Stain in Van Gieson's microfuchsin for two minutes (time will vary with age of solution).
8. Remove from staining solution and carefully place onto filter paper and blot dry.
9. Dehydrate through 95 % ethanol (couple of dips) and absolute ethanol (2 minutes).
10. Clear trough xylol or xylol substitute.
11. Mount with Permount.

<u>Results:</u>	Collagen	- bright red
	Other tissues	- yellow
	Reticulin	- unstained
	Nuclei	- blue/black
	Amyloid	- khaki color

Notes on the Procedure:

Nuclear Counterstain - Picric acid will decolorize Mayer's and Harris' haematoxylin considerably. Weigert's iron haematoxylin may be used for 10 minutes instead, but best of all is celestine blue followed by Mayer's hemalum. This gives a nuclear stain which resists phosphotungstic acid and phosphomolybdic acid and is relatively resistant to aqueous picric acid.

Hydrochloric acid - The addition of small amounts of acid to this stronger fuchsin solution, sharpens differentiation (collagen deeper red and muscle purer yellow).