Instructions for use

Van Gieson‘s Solution

3925

Van Gieson Trichrome Staining

This staining method allows a differential visualisation of tissue structures in paraffin sections.

Van Gieson’s solution contains two dyes with very different properties: The fine-particle picric acid infiltrates quickly all structures of tissue by staining them yellow. The coarse-particle acid fuchsine can stain only the coarse structures of collagen connective tissue during the short residence time. There the picric acid is masked. Do not prolong the residence time to avoid the masking of picric acid in other tissue structures, too (principle of progressive staining).
After staining remove the picric acid as completely as possible from collagen connective tissue for tissue stained with acid fuchsine tends to fade out when being exposed to acids and bases.
The procedure demands some skill for you have to stop rinsing before the picric acid is also removed from the other tissue structures (in that case the tissue becomes reddish).

The nuclei are stained with *Weigert’s iron hematoxylin solution*. The solution is acid resistant and, therefore, resistant against picric acid.

Additional chemicals required:

- Iron hematoxylin solution according to Weigert
  (Solution A, Art. No. X906, solution B, Art. No. X907)
- Ethanol denatured: 99.8 % (Art. No. K928), 96 % (T171), 70 % (T913)
- HCl-ethanol solution 3 % (Art. No. 6477) – working solution 0.5 %
- Usual Clearing Agents: Roti®-Histol (Art. No. 6640)
  Roticlear® (Art. No. A538)
  Xylene p.a. (Art. No. 4436)
- Appropriate Mounting Media: Roti®-Histokitt (Art. No. 6638), compatible with Roti®-Histol
  Roti®-Mount (Art. No. HP68), compatible with Roticlear®
  Roti®-Histokitt II (Art. No. T160), compatible with Xylene

Instruction*:

1. De-wax and rehydrate sections (descending alcohol series finishing off with ethanol 70 %).	7. Stain with van Gieson‘s solution. 1-3-min
2. Stain with iron hematoxylin solution acc. to Weigert (Mix solution A + B at a ratio of 1:1, solution stable for 8 days at room temp.). 5-10 min	8. Rinse shortly with ethanol 70% and ethanol 96%. *Caution, picric acid is especially soluble in diluted ethanol!*
3. Rinse with distilled water to avoid precipitation of hematein.	9. Dehydrate and rinse with ethanol 96%, finish with 2 x ethanol 100%.
4. *Examine by microscope: Nuclei should be grey blue, cytoplasm colorless to max. light grey.* If the cytoplasm is stained too intensive differentiate in HCl-ethanol 0.5%. 2-3 sec	10. Clear with clearing agent.
	11. Mount with appropriate mounting medium.
5. Rinse in tap water to interrupt the differentiation.	**Please note at step 9:** *Rinse moderately with highly concentrated ethanol to remove the picric acid from the connective tissue (see also above).*
6. Blue in flowing tap water. 10 min	

*Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result:

- Cell nuclei: dark blue/dark brown
- Collagene fibres: red
- Cytoplasm: yellow

Elastica van Gieson Staining

Van Gieson trichrome staining is well combinable with elastica staining acc. to Weigert allowing a good overview of various tissue structures, especially a differentiated visualisation of connective tissue and elastic fibres.

Additional chemicals required:

- Iron hematoxylin solution according to Weigert
  (Solution A, Art. No. X906, solution B, Art. No. X907)
- Ethanol denatured: 99.8 % (Art. No. K928), 96 % (T171), 70 % (T913)
- Resorcinol fuchsine solution according to Weigert (Art. No. X877)
- Usual Clearing Agents: Roti®-Histol (Art. No. 6640)
  Roticlear® (Art. No. A538)
  Xylene p.a. (Art. No. 4436)
- Appropriate Mounting Media: Roti®-Histokitt (Art. No. 6638), compatible with Roti®-Histol
  Roti®-Mount (Art. No. HP68), compatible with Roticlear®
  Roti®-Histokitt II (Art. No. T160), compatible with Xylene

Instruction*:

1. De-wax and rehydrate sections (descending alcohol series finishing off with ethanol 80 %).	9. Rinse with distilled water to avoid precipitation of hematein.
2. Stain with resorcinol fuchsine solution. 20-30 min	10. Blue in flowing tap water. 10 min
3. Rinse with tap water until stain fades.	11. Stain with van Gieson‘s solution. 1-3-min
4. Rinse with distilled water.	12. Rinse shortly with ethanol 70% and ethanol 96%. *Caution, picric acid is especially soluble in diluted ethanol!*
5. Differentiate with ethanol 80%.	13. Dehydrate and rinse with ethanol 96%, finish with 2 x ethanol 100%.
6. Rinse with distilled water to interrupt the differentiation.	14. Clear with clearing agent.
7. Examine by microscope: Elastic fibres dark violet, background light rose.	15. Mount with appropriate mounting medium.
8. Stain with iron hematoxylin solution acc. to Weigert (Mix solution A + B at a ratio of 1:1, solution stable for 8 days at room temp.). 2-3 min	**Please note at step 13:** *Rinse moderately with highly concentrated ethanol to remove the picric acid from the connective tissue and, therefore, avoid fading of the staining. Caution: If the rinsing is too intensive the tissue becomes reddish!*

*Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result:

- Elastic fibres: dark violet
- Cell nuclei: dark blue/dark brown
- Collagene fibres: red
- Muscle, cytoplasm: yellow

Please note:
The colour intensity depends on the pre-treatment and the composition of the samples to be stained. It may initially be necessary to adapt the method to the respective conditions.

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Van Gieson’s solution	**3925.1**	**500ml**
	3925.2	**1l**

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