
ELASTIC TISSUE FIBERS - VERHOEFF'S VAN GIESON (EVG)

PURPOSE: This stain is useful in demonstrating atrophy of elastic tissue in cases of emphysema, and the thinning and loss of elastic fibers in arteriosclerosis, and other vascular diseases. With increasing age, changes such as splitting and fragmentation occur, these changes are most obvious in the skin which becomes wrinkled and rather 'loose-fitting'.

PRINCIPLE: The tissue is stained with a regressive hematoxylin, consisting of ferric chloride and iodine. The differentiating is accomplished by using excess mordant (ferric chloride) to break the tissue-mordant dye complex. The dye will be attracted to the larger amount of mordant in the differentiating solution and will be removed from the tissue. The elastic tissue has the strongest affinity of the iron-hematoxylin complex and will retain the dye longer than the other tissue elements.

CONTROL: Artery or skin.

FIXATIVE: Any well fixed tissue.

TECHNIQUE: Cut paraffin sections 4 μ or 5 μ .

EQUIPMENT: Rinse glassware in DI water: coplin jars, graduated cylinders

REAGENTS:

Alcoholic Hematoxylin:

Hematoxylin	5.0 gm
Absolute alcohol	100.0 ml

Dissolve hematoxylin into alcohol with the aide of gentle heat, do not boil. Label with date and initials, solution is stable for 1 year.

CAUTION: Flammable, avoid contact and inhalation.

10% Ferric Chloride:

Ferric chloride	10.0 gm
Distilled water	100.0 ml

Mix well. Label with date and initials, solution is stable for 1 year.

CAUTION: Avoid contact and inhalation

Lugol's Iodine:

See Stock Solutions

CAUTION: Avoid contact and inhalation

CONNECTIVE TISSUE
ELASTIC STAIN - EVG

Verhoeff's Hematoxylin:

Alcoholic hematoxylin	20.0 ml
10% ferric chloride	8.0 ml
Lugol's iodine	8.0 ml

Add in the order given, mixing between additions. Make fresh, discard.

Caution: Avoid contact and inhalation.

**Differentiating Solution:
(2% Ferric Chloride)**

10% ferric chloride	10.0 ml
Distilled water	40.0 ml

Make fresh, discard.

CAUTION: Avoid contact and inhalation

5% Hypo:

See Stock Solutions

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Verhoeff's hematoxylin for 30 minutes (save solution until stain is completed)
3. Wash in tap water.
4. Differentiate in 2% ferric chloride solution, check microscopically for black fibers on a gray background.
5. Rinse in water.
6. Hypo for 1 minute to remove iodine.
7. Wash in water.
8. Counterstain in Van Gieson's for 5 minutes.
9. Dehydrate, clear in xylene, and coverslip.

RESULTS:

Elastic fibers and nuclei	black
Collagen	red
Other tissue elements	yellow

Van Gieson's Solution:

1% Acid fuchsin	1.0 ml
Picric acid, saturated	45.0 ml

Mix well. Allow to stand overnight. Label with date and initials. Stable for 2 weeks.

Caution: Toxic, avoid contact and inhalation.

SAFETY: Wear gloves(nitrile), goggles and lab coat. Work in well ventilated area, use fume hood, avoid contact and inhalation.

Picric acid: toxic, can become explosive if allowed to become dry.

Iodine: dermal sensitizer, irritant to eyes, skin and respiratory system.

REFERENCES:

- Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 194, Battelle Press, Ohio
- Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982, pp 125, 139, Churchill Livingstone, NY
- Luna L, Manual of Histologic Staining Methods of the AFIP, 3rd Ed, 1968, pp 76, McGraw Hill, NY
- Carson F, Histotechnology: A Self-Instructional Text, 1990, pp 147-149, ASCP, ILL
- Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

ELASTIC TISSUE FIBERS - VERHOEFF'S VAN GIESON (EVG)

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Verhoeff's hematoxylin for 30 minutes (save solution until stain is completed).
3. Wash in tap water.
4. Differentiate in 2% ferric chloride solution, check microscopically for black fibers on a gray background.
5. Rinse in water.
6. Hypo for 1 minute to remove iodine.
7. Wash in water.
8. Counterstain in Van Gieson's for 5 minutes.
9. Dehydrate, clear in xylene, and coverslip.

RESULTS:

Elastic fibers and nuclei	black
Collagen	red
Other tissue elements	yellow

NOTES:

1. The counterstain will remove some of the hematoxylin.
2. If over differentiated, place back into hematoxylin solution.

Alcoholic Hematoxylin:

Hematoxylin	5.0 gm
Absolute alcohol	100.0 ml

Dissolve hematoxylin into alcohol with the aide of gentle heat, do not boil .

Label with date and initials, solution is stable for 1 year.

CAUTION: Flammable

10% Ferric Chloride:

Ferric chloride	10.0 gm
Distilled water	100.0 ml

Mix well. Label with date and initials, solution is stable for 1 year.

Lugol's Iodine:

See Stock Solutions

5% Hypo:

See Stock Solutions

Verhoeff's Hematoxylin:

Alcoholic hematoxylin	20.0 ml
10% ferric chloride	8.0 ml
Lugol's iodine	8.0 ml

Add in the order given, mixing between additions. Make fresh, discard.

Differentiating Solution: (2% Ferric Chloride)

10% ferric chloride	10.0 ml
Distilled water	40.0 ml

Make fresh, discard.

Van Gieson's Solution:

1% Acid fuchsin	1.0 ml
Picric acid, saturated	45.0 ml

Mix well. Allow to stand overnight. Label with date and initials. Stable for 2 WEEKS.

CAUTION: Corrosive

VAN GIESON'S SOLUTION:

1% Acid fuchsin 1.0 ml
Picric acid, saturated 45.0 ml

Mix well. Allow to stand overnight.
Label with date and initials.
Solution is stable for 2 WEEKS

CAUTION: Toxic, avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

VAN GIESON'S

DATE: _____

TECH: _____

ALCOHOLIC HEMATOXYLIN:

Hematoxylin 5.0 gm
Absolute alcohol 100.0 ml

Dissolve hematoxylin into alcohol with the aide of gentle heat, do not boil. Label with date and initials. solution is stable for 1 year.

CAUTION: Flammable, avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

10% FERRIC CHLORIDE:

Ferric chloride 10.0 gm
Distilled water 100.0 ml

Mix well. Label with date and initials, solution is stable for 1 year.

Caution: Avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____