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VERHOFF - VAN GIESON STAIN PROTOCOL (VVG)

PRINCIPLE:

The first step in the procedure is an overstaining of the tissue section with a soluble lake of hematoxylin-ferric chloride-iodine. Ferric chloride and iodine both serve mordant functions primarily and oxidizing functions secondarily. The latter characteristic will assist in the conversion of hematoxylin dye to hematein. In addition, the iodine may serve as a dye-trapping agent, thereby retarding dye loss from selected components during the subsequent differentiation process. Differentiation, a necessary step in any overstaining process, is accomplished by use of excess mordant (a dilute solution of ferric chloride) to break the tissue-mordant-dye complex. The elastic tissues, having the strongest affinity for the insoluble complex, retain it the longest and so are colored black in the final result. The van Gieson solution of acid fuchsin and picric acid is used as the counterstain and colors the collagen bright red and other tissue elements yellow.

QUALITY ASSURANCE:

The period of differentiating is important and somewhat difficult to control as the counterstaining will further differentiate the stain. Empirically the differentiating period that seems to offer the best results has been determined to be about five minutes.

SPECIMEN REQUIRED:

Snap frozen human striated muscle. (Use the 2-methylbutane freezing method previously described.)

METHOD:

Fixation: Use snap frozen tissue. Fix the sections just before staining.

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No.1½, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps Latex gloves

Reagents:

Absolute alcohol (100% ethanol) - Quantum, **FLAMMABLE**
Store at room temperature in a flammable cabinet
Calcium Chloride, anhydrous - Sigma C4901,
Store at room temperature
Iron (III) Chloride, hexahydrate, lump (Sigma-Aldrich 236489)
HEALTH HAZARD, USE IN HOOD
Formaldehyde, 37 % - Fisher F79-500, **POISON,**
CARCINOGEN, store at room temperature

Fuchsin acid - Fisher F-97, store at room temperature
Hematoxylin, Sigma-Aldrich H3136, store at room temperature
Iodine, crystalline - Sigma I-0385, **CORROSIVE, NOXIOUS VAPOR**, store at room temperature
Permount - Fisher SP15-100, **FLAMMABLE HEALTH HAZARD**
Picric acid - Fisher A-253, **FLAMMABLE, EXPLOSIVE WHEN DRY OR IN CONTACT WITH METALS !!,**
Store room temperature as a water saturated solution.
Potassium Iodide - Sigma P8256, **TERATOGEN**,
Store at room temperature
Reagent alcohol, ACS - histochemical Fisher A962-4 or HPLC A995,
FLAMMABLE, TOXIC, TERATOGENIC,
Store at room temperature in flammable cabinet
Xylenes - Fisher #HC700-1GAL,
FLAMMABLE, store room temperature in flammable cabinet

Solutions:

1. BAKER'S FIXATIVE (modified calcium-formol)

Calcium Chloride, anhydrous ($CaCl_2$)		0.75 g
Formaldehyde, 37%(HCHO)		7.5 ml
deionized water	→	250 ml

store at room temperature

2. FERRIC CHLORIDE SOLUTION, 10 %

ferric chloride ($FeCl_3 \bullet 6H_2O$)	0.5 g
deionized water	5.0 ml

3. FERRIC CHLORIDE SOLUTION, 1 %

ferric chloride solution, 10 %	1 ml
deionized water	9 ml

4. LUGOL'S IODINE SOLUTION **(store** at room temp. **in an amber bottle)**

Potassium Iodide (KI)	1 g
dissolve in deionized water	1 ml
when completely dissolved	
add iodine crystals (I_2)	0.5 g
when iodine is completely dissolved	
add D.I. H_2O to a final volume	25 ml

5. VERHOFF'S STAINING SOLUTION **(PREPARE FRESH FOR EACH STAIN)**

hematoxylin, dark	0.25 g
ethanol, 100 %	5.0 ml

when dissolved add in the order given:

ferric chloride solution, 10 %	2.0 ml
mix well	
Lugol's Iodine solution	2.0 ml

6. PICRIC ACID, SATURATED AQUEOUS SOLUTION

picric acid	~ 15 g
deionized water	500 ml

Store at room temperature **in an amber bottle in fume hood**

7. VAN GIESON'S STAINING SOLUTION

Acid Fuchsin	100 mg
Saturated Picric Acid Solution	100 ml

store at room temperature

8. ALCOHOL 50 %

reagent alcohol	~50 ml
deionized water	~50 ml

9. ALCOHOL 70 %

reagent alcohol	~70 ml
deionized water	~30 ml

10. ALCOHOL 80 %

reagent alcohol	~80 ml
deionized water	~20 ml

11. ALCOHOL 95 %

reagent alcohol	~95 ml
deionized water	~ 5 ml

Staining Procedure:

1. Place coverslips with sections in Baker's Solution in a columbia staining dish (Thomas Scientific #8542-C12) for 10 minutes at room temperature.

2. Wash with three exchanges of tap or deionized H_2O.

3. Add Verhoff's Staining Solution to dish for 20 minutes at room temperature.

4. Rinse quickly with tap water to remove most of the Verhoff stain**. (NOTE: sections will be too blue if left in contact with water for more than a very short time.)**

5. Add 1 % Ferric Chloride for up to 5 minutes (sections should still be dark).

6. Rinse quickly with tap water to remove excess 1 % Ferric Chloride.

7. Immediately add van Gieson's Stain for 2 minutes.

8. Rinse quickly with tap water to remove excess stain.

9. Do not leave in water and immediately transfer to ceramic rack (Thomas Scientific #8542-E40).

10. Dehydrate in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, 100% x 2) in Columbia staining dishes - Thomas Scientific #8542-E30.

11. Clear with xylene (3 - 4 x) also in columbia staining dish(jar)s - Thomas Scientific #8542-E30.

12. Mount coverslip onto a labeled glass slide with Permount or some other suitable organic mounting medium.

Results:

Nuclei → blue to black
Normal muscle myofibrils → tan to brown (the two major fiber types are distinguishable)
Connective tissue fibrils → blue-black to black
Collagen → red to purple

REFERENCES:

1. Thompson, Samuel W. *SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS,* Charles C. Thomas, Springfield, IL, 1966.

2. Sheehan, D.C. and Hrapchak, B.B., *THEORY AND PRACTICE OF HISTOTECHNOLOGY,* 2nd Edition; Battelle Memorial Institute, Columbus, OH, 1987.

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