ELASTIC TISSUE - MODIFIED ELASTIC TRICHROME STAIN

**PURPOSE:** To differentiate different types of connective tissue, in a single tissue section.

**PRINCIPLE:** Verhoff’s Hematoxylin stains the elastic fibers, and a modified Masson’s Trichrome is used to stain the other connective tissue elements.

**FIXATIVE:** 10% formalin or Zenker’s

**TECHNIQUE:** Cut paraffin sections 4-5m.

**EQUIPMENT:** Microwave, coplin jars (rinse in DI water)

**REAGENTS:**

- **Weigert’s Iodine:** See Microwave Trichrome
- **5% Hypo**
  See Stock Solutions
- **Zenker’s Fluid:**
  Distilled water  500.0 ml
  Mercuric chloride  25.0 gm
  Potassium dichromate  12.5 gm
  Sodium sulfate  5.0 gm
  Mix well, label with date and initial. Solution is stable indefinite.
- **Working Solution:** Right before use, to 50 ml of Zenker’s add 5 ml glacial acetic acid. Discard after use.
- **CAUTION:** Corrosive poison, discard in labeled glass container.

- **Verhoeff’s Hematoxylin:**
  5% Alcoholic hematoxylin  20.0 ml
  10% ferric chloride  8.0 ml
  Lugol’s iodine  8.0 ml
  Add in order given, make right before use, discard when stain is completed.
  **Caution:** Flammable, avoid contact and inhalation.

- **Biebrich Scarlet:**
  See Masson’s Trichrome

- **Phosphotungstic / phosphomolybdic Acid:**
  See Masson’s Trichrome

- **Aniline Blue:**
  See Masson’s Trichrome

- **1% Acetic Acid:**
  See Masson’s Trichrome
SAFETY: Wear nitrile gloves and lab coat, wear a particle mask or work under the hood. Keep all hot uncapped solutions under the hood. Avoid contact and inhalation.

Zenker’s contains; Mercuric chloride: Severe skin and eye irritant; target organ effects on reproductive, urogenital, respiratory, gastrointestinal and fetal systems via ingestion and inhalation routes.

Potassium dichromate: toxic by inhalation of dust and by ingestion; target organ effects on reproductive and fetal systems. Solid form is corrosive to eyes, skin and mucous membranes.

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

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

PROCEDURE:
1. Deparaffinize and hydrate to water.
2. Mordant in Weigert’s iodine, microwave 45 seconds.
3. Rinse in water.
4. Decolorize in 5% hypo solution, wash in water.
5. Mordant in Zenker’s, microwave 60 seconds.
6. Wash in tap water.
7. Verhoeff’s hematoxylin for 20-30 minutes.
8. Wash in water.
9. Hypo for 3-5 minutes.
10. Wash in water.
11. Biebrich scarlet, microwave 45 seconds.
12. Rinse in distilled water.
13. Phosphotungstic/phosphomolybdic acid, microwave 45 seconds.
15. Aniline blue, microwave 10 seconds.
16. Rinse in distilled water.
17. Acetic acid rinse for 1 minute.
18. Dehydrate quickly through alcohols, clear in xylene and coverslip.

RESULTS:
Elastic fibers and nuclei: black
Collagen: blue
Cytoplasm, muscle fibers, keratin, and fibrin: red
CONNECTIVE TISSUE
MODIFIED ELASTIC TRICHROME STAIN

REFERENCES:
University of Utah Histology Laboratory S Q
Modified Movat’s Stain.
Crookham,J, Dapson,R, Hazardous Chemicals in the Histology Laboratory,
2nd ED, 1991, Anatech

Prepared:_________________________ By: ________________

Approved:_______________________ By:__________________

Downloaded from WebPath: Internet Pathology Laboratory
http://www-medlib.med.utah.edu/WebPath/webpath.html
PROCEDURE CARD

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RESULTS:
cytoplasm, muscle fibers, keratin, fibrin: red collagen: blue
Elastic fibers, nuclei: black

SAFETY: Wear gloves and lab coat, work under the hood or in a well ventilated room. Do not discard Zenkers fix down the drain, it contains mercury. Dispose through Safety Services.

Weigert’s Iodine:
See Microwave Trichrome

5% Hypo
See Stock Solutions

Zenkers Fluid:
Working Solution: right before use, to 50 ml of Zenkers add 5 ml glacial acetic acid. Discard after use.

CAUTION: Corrosive, poison

Phosphotungstic/phosphomolybdic Acid:
See: Masson’s Trichrome

Analine Blue:
See Masson’s Trichrome

1% Acetic Acid:
See Masson’s Trichrome

Verhoeff’s Hematoxylin:
Alcoholic hematoxylin 20.0 ml
10% ferric chloride 8.0 ml
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Biebrich Scarlet:
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ZENKERS FLUID:
Distilled water 500.0 ml
Mercuric chloride 25.0 gm
Potassium dichromate 12.5 gm
Sodium sulfate 5.0 gm

Mix well, label with date and initial. Solution is stable indefinite.

Working Solution:
Zenkers 50.0 ml
Acetic acid 5.0 ml

Mix right before use. Discard after use.

CAUTION: Corrosive poison, discard in labeled glass containers

DATE:________________________

TECH:________________________

EXPIRATION:__________________