GLIAL FIBERS - HOLZER'S STAIN

PURPOSE: An appropriate term for glial fibers is 'nerve glue', because they provide the internal support of the central nervous system. There are four types of glial cells: astrocytes, oligosendroglia, microglia, and ependymal cells.

PRINCIPLE: The glia fibers are stained with crystal violet which are resistant to the aniline-chloroform differentiating solution.

CONTROL: Cerebral cortex

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections at 6µ.

EQUIPMENT: Rinse all glassware in DI water, coplin jars, bibulous paper.

REAGENTS:
Phosphomolybdic Alcohol:
0.5% phosphomolybdic acid 20.0 ml
95% alcohol 40.0 ml
Make fresh, discard after use.

CAUTION: flammable, avoid contact and inhalation.

Absolute Alcohol - Chloroform:
Absolute alcohol 40.0 ml
Chloroform 160.0 ml
Make fresh, discard after use.

CAUTION: Carcinogen, flammable.

10% Potassium Bromide:
Potassium bromide 10.0 gm
Mix well. Solution is stable for 3 months.

Holzer’s Crystal Violet:
Crystal violet 5.0 gm
Absolute alcohol 20.0 ml
Chloroform 80.0 ml
Mix well. Solution is stable for 3 months.

CAUTION: Carcinogen, flammable.

CAUTION: Avoid contact and inhalation.

Differentiating Solution:
Aniline oil 30.0 ml
Chloroform 45.0 ml
Ammonium hydroxide 5 drops
Make fresh, discard after use.

CAUTION: Carcinogen, flammable, corrosive.
SAFETY: Work in fume hood, wear nitrile gloves, goggles and lab coat. Avoid contact and inhalation.

Phosphomolydic acid; irritant to skin and eyes.

Chloroform; carcinogen. Toxic on ingestion and inhalation. Target organ effects on liver, reproductive system, fetal tissue, CNS, blood and GI systems. Overexposure to vapor can cause unconsciousness and death.

Crystal violet (Gentian violet); carcinogen.

Aniline; possible carcinogen, neoplastic. Moderate skin, severe eye irritant. Sensitizer. Toxic by skin absorption. Excessive exposure may cause blue discoloration of extremities, nausea, headache and drowsiness.

PROCEDURE:
1. Deparaffinize and hydrate to 95% alcohol.
2. Phosphomolybdic acid solution, 3 minutes.
3. Drain off solution.
4. Alcohol-chloroform solution until sections are translucent, drain.
5. Crystal violet stain, 30 seconds.
6. Drain off solution.
7. Potassium bromide solution, 5 minutes.
8. Wash in distilled water, blot dry with bibulous paper.

RESULTS:
Glia fibers deep violet-blue
Background pale violet
REFERENCES:
Carson F, Histotechnology: A Self-Instructional Text, 1990, pp. 177-178, ASCP, Ill
Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: ______________________ By: ______________________

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PROCEDURE CARD

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RESULTS:
- Glia fibers: deep violet-blue
- Background: pale violet

Phosphomolybdic Alcohol:
- 0.5% phosphomolybdic acid: 20.0 ml
- 95% alcohol: 40.0 ml
Make fresh, discard after use.

10% Potassium Bromide:
- Potassium bromide: 10.0 gm
- Distilled water: 100.0 ml
Mix well. Solution is stable for 3 months.

CAUTION: Flammable, avoid contact and inhalation.

Absolute Alcohol - Chloroform:
- Absolute alcohol: 40.0 ml
- Chloroform: 160.0 ml
Make fresh, discard after use.

CAUTION: Carcinogen, flammable.

Differentiating Solution:
- Aniline oil: 30.0 ml
- Chloroform: 45.0 ml
- Ammonium hydroxide: 5 drops
Make fresh, discard after use.

CAUTION: Carcinogen, flammable, corrosive.

Holzer’s Crystal Violet:
- Crystal violet: 5.0 gm
- Absolute alcohol: 20.0 ml
- Chloroform: 80.0 ml
Mix well. Solution is stable for 3 months.

CAUTION: CARCINOGEN, FLAMMABLE
10% Potassium Bromide:
Potassium bromide       10.0 gm
Distilled water       100.0 ml

Mix well. Solution is stable for 3 months.

**CAUTION: Avoid contact and inhalation.**

DATE:__________________________

TECH:__________________________

EXPIRATION:

Holzer’s Crystal Violet:
Crystal violet       5.0 gm
Absolute alcohol   20.0 ml
Chloroform         80.0 ml

Mix well. Solution is stable for 3 months.

**CAUTION: CARCINOGEN, FLAMMABLE**

DATE:__________________________

TECH:__________________________

EXPIRATION:____________________