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**GLIAL FIBERS - HOLZER'S STAIN**

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**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, oligodendroglia, 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 6 $\mu$ .

**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.

Phosphomolybdic 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.
9. Differentiating solution, check microscopically.
10. Clear in xylene, coverslip.

**RESULTS:**

Glia fibers	deep violet-blue
Background	pale violet

**REFERENCES:**

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 258-260, Battelle Press, Ohio

Carson F, Histotechnology: A Self-Instructional Text, 1990, pp. 177-178, ASCP, III

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

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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.**

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**Absolute Alcohol - Chloroform:**

Absolute alcohol	40.0 ml
Chloroform	160.0 ml

Make fresh, discard after use.

**Differentiating Solution:**

Aniline oil	30.0 ml
Chloroform	45.0 ml
Ammonium hydroxide	5 drops

Make fresh, discard after use.

**CAUTION: Carcinogen, flammable.**

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**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
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