PATHOLOGY CORE

Special Stain Protocol: Kluver-Barrera (Luxol Fast Blue)

Purpose:
The purpose is to detect myelin fibers.

Principle:
Since the technic is usually done on paraffin sections, lipoproteins, rather than simple lipids, are responsible for the staining. The mechanism is one of an acid-base reaction with salt formation, because the base of the lipoprotein replaces the base of the dye.

Positive Control Tissue:
Brain tissue

Tissue Fixative:
10% Formalin fixed tissue

Reagents Required:
Ethanol
- Vendor – Epredia
- Catalog number – 6201

Luxol Fast Blue (Solvent Blue 38)
- Vendor – Sigma Aldrich
- Catalog number – S3382-25G

Cresyl Echt Violet
- Vendor – Poly Scientific R&D Corp
- Catalog number – s167C-16OZ

Lithium Carbonate
- Vendor – Fisher Chemical
- Catalog number – L119-500

Acetic Acid
- Vendor - Fisher Chemical
- Catalog number – A38-500

Solution Preparation:
0.1% Luxol Fast Blue: 100mL of 95% Ethanol + 0.1g of Luxol Fast Blue. Add 0.5mL of Glacial Acetic Acid for each 100mL

0.1% Cresyl Echt Violet Solution: Just before using, add 15 drops of 15% glacial acetic acid then filter.

0.05% Lithium Carbonate Solution: 100mL of distilled water + 0.05g of Lithium Carbonate

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Protocol:

1. Deparaffinize the slides and hydrate to 95% Alcohol (xylene→95% Alcohol)
2. Luxol fast blue solution at 560-600C overnight
3. Rinse in 95% alcohol to remove excess stain
4. Rinse in distilled water
5. Begin differentiation by quick immersion in lithium carbonate solution
6. Continue differentiation in 70% alcohol solution until gray and white matter can be distinguished
7. Wash with distilled water
8. Finish differentiation by rinsing briefly in lithium carbonate solution and then putting through several changes of 70% alcohol until the greenish blue of the white matter contrasts sharply with the colorless gray matter
9. Rinse thoroughly in distilled water
10. Filter and preheat Cresyl Echt violet to 570C just before use
11. Cresyl Echt violet solution for 10
12. minutes
13. Differentiate in several changes of 95% alcohol (add a few drops of 5N HCL to speed up differentiation)
14. Dehydrate in absolute alcohol, clear in xylene, and mount in a synthetic resin medium

Interpretation:

Myelin – Blue
Cell Products – Pink to Violet

References:

Theory and Practice of Histotechnology