PATHOLOGY CORE

Special Stain Protocol: Gordon and Sweet's Reticulin Method

Purpose:
The purpose is to detect reticular connective tissue, or reticulin.

Principle:
The reticulin fibers are first oxidized by the potassium permanganate. It is then sensitized using an iron alum solution that targets and binds to the reticulin fibers. The fibers are then impregnated by the silver solution that removes and replaces the sensitizer. The silver solution is reduced by 10% formalin so that a visible metallic tone highlights the reticulin fibers. The metallic silver is then toned and converted to metallic gold using gold chloride solution, thereby providing better chemical stability, fiber contrast, and clarity. Unreduced silver and excess gold are removed via a 5% sodium thiosulfate solution. The tissue section may then be counter-stained with nuclear fast-red.

Positive Control Tissue:
Liver tissue

Tissue Fixative:
10% Formalin fixed tissue

Reagents Required:
Silver Nitrate
- Vendor – Fisher Chemical
- Catalog number – S181-25

Ammonium Hydroxide
- Vendor – Fisher Chemical
- Catalog number – A669-500

Sodium Hydroxide
- Vendor – Fisher Scientific
- Catalog number – S318-100

Potassium Permanganate
- Vendor – Sigma-Aldrich
- Catalog number – P-2097

Sulfuric Acid
- Vendor – Fisher Chemical
- Catalog number – A300-500

Oxalic Acid
- Vendor – Sigma-Aldrich
- Catalog number – O0376-100G

Iron Alum(Ferric Ammonium Sulphate)
- Vendor – Fisher Chemical
- Catalog number – I75-500

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10% Neutral Formalin
- Vendor – Fisher Scientific
- Catalog number – 23-730-582

Gold Chloride
- Vendor – Poly Scientific R&D
- Catalog number – 3202-807

Sodium Thiosulphate
- Vendor – Fisher Scientific
- Catalog number – S446-500

Nuclear Fast-red (Kenechtrot)
- Vendor – VWR
- Catalog number – 20E2856739

Pyridine
- Vendor – Fisher Chemical
- Catalog number – P368-500

Solution Preparation:
Silver Solution: To 5mL of Silver Nitrate, add strong ammonia drop by drop until the resulting precipitate is just dissolved. Add 5mL of 3.1% sodium hydroxide and redissolve the precipitate with a few more drops of ammonia. Dilute to 50mL with distilled water.

Protocol:
1. Deparaffinize the slides (xylene→distilled water)
2. Oxidize for 1-5 minutes in 0.5% potassium permanganate, 47.5mL, 3% sulfuric acid, 2.5mL
3. Wash briefly in water
4. Bleach in 1% Oxalic Acid
5. Rinse in distilled water followed by thorough washing in tap water
6. Sensitize in 2.5% iron alum for 15 minutes (the iron alum may be used repeatedly)
7. Wash thoroughly with distilled water 2 or 3 times
8. Cover with the silver solution for 30 seconds, or until sections become transparent
9. Wash well with distilled water
10. Reduce with 10% neutral formalin for 2 minutes
11. Wash in tap water followed by distilled water
12. Tone in 0.2% gold chloride for 2 minutes (the sections turn a purplish color)
13. Wash briefly with distilled water
14. Fix in 5% sodium thiosulphate for 5 minutes
15. Wash well in water
16. Counterstain nuclei with nuclear fast-red
17. Dehydrate, clear, and mount in a synthetic resin medium

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Interpretation:
Reticulin Fibers – Black
Collagen and Cytoplasm – Brown to yellow-brown if untoned, purplish-grey if toned
Nuclei – As counterstain

References:
Staining Manual Wills Eye Hospital
https://www.labce.com/spg531604_gordon_and_sweets_silver_staining___chemistry.aspx