
VON KOSSA'S METHOD - CALCIUM

PURPOSE: Abnormal deposits of calcium may be found in any area of the body. With the H&E stain, calcium appear deep blue-purple.

PRINCIPLE: Tissue sections are treated with silver nitrate solution, the calcium is reduced by the strong light and replaced with silver deposits, visualized as metallic silver.

CONTROL: Tissue containing known positive calcium deposits, or undecalcified bone.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections at 4 μ .

EQUIPMENT: Acid cleaned glassware, 60-watt lamp, foil or mirror.

REAGENTS:

5% Silver Nitrate Solution:

Silver nitrate 25.0 gm
Distilled water 500.0 ml

Mix well, pour into acid clean brown bottle. Store in the refrigerator. Solution is stable for 1 year.

5% Hypo

CAUTION: Avoid contact and inhalation.

Nuclear Fast Red:

See Retic

CAUTION: Avoid contact and inhalation.

SAFETY: Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Silver nitrate; Severe skin and eye irritant. Ingestion will produce violent GI distress. Tumorigenic. Oxidizer.

Hypo (sodium thiosulfate); Powder is an eye, skin and respiratory irritant. Ingestion may cause GI distress.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. 5% Silver solution, place in bright sunlight, or in front of a 60-watt lamp, place foil (or mirror) behind the jar to reflect the light. Leave for 1 hour or until calcium turns black.
3. Rinse in distilled water, 3 changes.
4. 5% Hypo, 5 minutes.
5. Wash in tap water, rinse in distilled.
6. Nuclear-fast Red, 5 minutes.
7. Wash in water.
8. Dehydrate, clear, and coverslip.

RESULTS:

Calcium salts	black
Nuclei	red
Cytoplasm	pink

REFERENCES:

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 226-227, Battelle Press, Ohio
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD
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NUCLEAR-FAST RED

DATE: _____

TECH: _____