FONTANA-MASSON SILVER METHOD - MELANIN

PURPOSE: To identify argentaffin granules and melanin. Melanin is a nonlipid, nonhematogenous pigment. It is a brown-black pigment present normally in the hair, skin, retina, iris and certain parts of the CNS. Argentaffin granules are found in carcinoid tumors.

PRINCIPLE: A positive argentaffin reaction means the cells take-up silver and then reduce it to a visible metallic state, without the aid of a reducing agent.

CONTROL: A nevi for melanin, or small intestine for argentaffin.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections 4μ.

EQUIPMENT: Acid cleaned glassware, microwave oven (or 60°C oven), microscope.

REAGENTS:

**Ammoniacal Silver Stock Solution:**
- 10% Silver nitrate 25.0 ml

Add ammonium hydroxidd drop by drop, until solution precipitates and clears again.

- 10% silver nitrate 1.0 ml

Solution will become slightly cloudy, allow to remain undisturbed for 4-24 hours. Discard after use.

**Ammoniacal Silver Working Solution:**
- Ammoniacal silver stock solution 12.5 ml
- Distilled water 37.5 ml

Pipette the ammoniacal silver off the top, leaving the precipitate on the bottom. Filter. Discard after use.

CAUTION: CORROSIVE - avoid contact and inhalation.
10% Silver Nitrate:
Silver nitrate 20.0 gm
Distilled water 200.0 ml
Mix well, store in acid cleaned brown bottle, in the refrigerator. Good for 6 months.

CAUTION: Avoid contact and inhalation.

0.1% Gold Chloride:
1% Gold Chloride, Stock 5.0 ml
Distilled water 45.0 ml

CAUTION: Avoid contact and inhalation.

5% Hypo:
CAUTION: Avoid contact and inhalation.

Nuclear-Fast Red:
See Retic

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

Silver nitrate: severe skin and eye irritant. Oxidizer. Ingestion will produce violent gastrointestinal discomfort. Possible carcinogen: equivocal tumorigenic agent.

Ammoniacal silver solution can be explosive, discard down the drain followed by copious amounts of running water.

Ammonium hydroxide: severe eye irritant, irritating to respiratory system. Target organ effects on respiratory system. Corrosive. Work under hood.

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.
PROCEDURE:
1. Deparaffinize and hydrate to distilled water.
2. *Working Silver Solution, microwave 20 power, 2 minutes. Check slides microscopically for adequate intensity, place in microwave a few seconds longer if needed.
3. Rinse in distilled water.
4. 0.1% Gold chloride, 10 minutes.
5. Rinse in distilled water.
6. 5% Hypo, five minutes.
7. Wash in tap water, rinse in distilled.
8. Nuclear-fast red, 5 minutes.
9. Wash in tap water.
10. Dehydrate, clear, and coverslip.
   *Conventional Method: Place in 60°C oven for 1 hour.

RESULTS:
Melanin, argentaffin cells black
Nuclei red

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

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Mix well, store in acid cleaned brown bottle, in the refrigerator.
Good for 6 months.
*CAUTION: Avoid contact and inhalation.*

DATE:_____________________
TECH:_____________________
EXPIRATION:_________________

**0.1% GOLD CHLORIDE:**
1% Gold Chloride, Stock 5.0 ml
Distilled water 45.0 ml
*CAUTION: Avoid contact and inhalation.*

DATE:_____________________
TECH:_____________________
EXPIRATION:_________________

**0.1% GOLD CHLORIDE**

DATE:_____________________
TECH:_____________________

**NUCLEAR-FAST RED**

DATE:_____________________
TECH:_____________________