
BIELSCHOWSKY TECHNIQUE - SENILE PLAQUES

PURPOSE: A silver stain to demonstrate neurofibrillary tangles, nerve fibers and senile plaques in Alzheimer's disease.

PRINCIPLE: The nerve fibers are sensitized with a silver solution. The sections are treated with ammoniacal silver, and then reduced to a visible metallic silver.

CONTROL: Cerebral cortex of a known case of Alzheimer's disease.

FIXATION: 10% Neutral buffered formalin.

TECHNIQUE: Paraffin sections, 8 μ , allow to dry overnight at 37°C.

EQUIPMENT: Acid cleaned glassware and coplin jars, pipettes, microwave oven and 37°C oven.

REAGENTS:

20% Silver Nitrate

Silver nitrate 10.0 gm
Distilled water 50.0 ml

Make fresh.

CAUTION: Skin irritant, avoid contact.

Ammoniacal Silver Solution

20% silver nitrate 50.0 ml
ammonium hydroxide

Add ammonia drop by drop until the precipitate formed has completely dissolved. Make right before use, discard after use.

CAUTION: Skin and respiratory system irritant, avoid contact and inhalation.

Developer

Stock Solution:

Formaldehyde (37-40%) 20.0 ml
Citric acid 0.5 gm
Nitric acid 2 drops
Distilled water 100.0 ml

Make fresh. Discard after use.

CAUTION: Carcinogenic, corrosive.

Working Solution:

Distilled water 50.0 ml
Ammonium hydroxide 8 drops
Developer, stock 8 drops

Make fresh just before use, discard.

CAUTION: Skin and respiratory system irritant, carcinogenic.

Ammonia Water: 5% Hypo

Distilled water 50.0 ml

Ammonium hydroxide 8 drops

Make fresh, discard.

CAUTION: Skin and respiratory system irritant, avoid contact and inhalation.

SAFETY: Wear gloves, goggles and lab coat. Work under fume hood, 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.

Formaldehyde: known carcinogen.

Nitric acid; Toxic on inhalation. May cause respiratory distress. Target organ effects on reproductive and fetal system.

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

PROCEDURE:

1. Hydrate to distilled water, 3 changes.
2. Preheat a coplin jar of 20% Silver nitrate solution, 35 seconds in the microwave, add slides, place in a 37°C oven for 15 minutes.
3. Wash in distilled water, 3 changes.
4. Place slides in Ammoniacal silver solution, 10 minutes, in 37°C oven.
5. Place slides in Working Developer solution, 1 to 5 minutes, check under microscope.
6. Wash in fresh ammonia water.
7. Wash in distilled water, 3 changes.

8. Place slides in 5% Hypo, 2 minutes.
9. Wash in distilled water, 3 changes.
10. Dehydrate, clear and mount.

RESULTS:

axons, plaque neurites and tangles:	black
background:	yellow to brown
plaque and vascular amyloid:	generally brown to dark brown

NOTE: The author suggests fresh solution every 10-15 slides.

REFERENCES:

- Mirra, SS, Hart, MN, Terry, RD: Making the Diagnosis of Alzheimer's Disease, Arch Pathol Lab Med. 1993;117:132-144
- Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

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