

BIELSCHOWSKY'S METHOD FOR NEUROFIBRILS

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of senile plaques and fibrillary tangles.
PRINCIPLE:	Nerve fibers are first sensitized with a Silver Solution and then they absorb silver from the Diamine Silver Solution. The silver is reduced to metallic silver by Formalin and the excess is removed by Thiosulfate.
CONTROL:	Cerebral cortex of a known cause of Alzheimer's <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs031.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Silver Nitrate 20% Aqueous Item# s2003 2. Ammonium Hydroxide Concentrated Item# c804 3. Ammonium Hydroxide 1% Solution Item# s2338 4. Sodium Thiosulfate 5% Aqueous Item# s1895 5. Bielchowsky's Developer Solution Item# s2788 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	Use acid cleaned glassware. Use the same silver solution throughout technique. Do not crowd slides in staining container.
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts</u> . American Histolabs Inc. Gaithersburg, MD. 1992. pp. 484-485.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place in 50 mL of prewarmed (37°C) Silver Nitrate 20% Aqueous for 10 minutes.
3. Place slides in distilled water.
4. To the Silver Nitrate 20% Solution add 6.0 mL of Ammonium Hydroxide Concentrated.
5. Clear the silver, which at this point is black, by adding, drop by drop, Ammonium Hydroxide Concentrated until the precipitate clears. Do not use more than 10 mL of Ammonium Hydroxide. Excess ammonia may cause a precipitate or result in a poor impregnation of the fibers.
6. Place slides in this Ammoniacal Silver Solution and stain for 10 minutes at 37°C.
7. Place slides in Ammonium Hydroxide 1% Solution for 1-3 minutes.
8. To the Ammoniacal Silver add 15 drops of the Bielchowsky's Developer Solution. Place slides in this solution for 3-5 minutes.
9. Dip slides in Ammonium Hydroxide 1% Solution to stop the silver reaction. Check microscopically.
10. If fibers are not dark enough, dip in Ammonium Hydroxide 1% Solution before returning to Ammoniacal Silver Developing Solution for 3 minutes.
11. Rinse in Ammonium Hydroxide 1% Solution for 3 minutes.
12. Pour on Sodium Thiosulfate 5% Aqueous and allow to remain for 5 minutes.
13. Rinse in 3 changes of distilled water, 5 minutes each.
14. Dehydrate and clear in 95% Alcohol, Absolute Alcohol and Xylene, 2 changes each for 2 minutes each.
15. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Neurofibrils and Senile Plaques Black
Background Yellow to Brown

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