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Bielschowsky Stain

OVERVIEW

An individual with Alzheimer's disease will exhibit twisting and clumping of neurons in the brain. This disease is seen in individuals 65 of age and is more prevalent in individuals over 85. This occurrence causes memory failure and inevitability affects the behavior of the individual. There are many research studies focused on aging and brain disorders, as well as, other neurological conditions that come from natural age progression. The Bielschowsky Stain is a very useful method in detecting the twist and clumping of neurons in the brain, otherwise known as, neurofibrillary tangles and senile plaques, which are signs of the Alzheimer's disease. The Bielschowsky stain, along with other diagnostic test, can assist scientist and researchers in the mission for finding a cure for this disease of memory loss.

FIXATION – 10% neutral buffered formalin

TECHNIQUE – Cut paraffin sections at 8 – 10 microns

KIT CONTAINS

SOLN A – 10% Silver Nitrate Solution
SOLN B – Ammonium Hydroxide, concentrate
SOLN C – Developer Stock Solution
SOLN D – 1% Ammonium Hydroxide Solution
SOLN E – 5% Sodium Thiosulfate

PROCEDURE

1. Preheat a container of 10% Silver Nitrate Solution to 37 – 40°C in the oven or waterbath. Allow reagent to remain in oven or waterbath until needed. Note: Check temperature using a glass thermometer.

2. Deparaffinize sections through two changes of xylene, 5 minutes each. Hydrate through two changes of 100% ethyl alcohol and 95% ethyl alcohol, 3 minute each. Rinse in DI water.

3. Place slides in pre-warmed (37 – 40°C) 10% Silver Nitrate Solution (SOLN A). Incubate for 15-20 minutes in the oven or waterbath at 37 – 40°C. Keep this reagent for future steps.

4. Rinse slides in 2 changes of DI water.

5. Obtain the container of pre-warmed 10% Silver Nitrate Solution (used in step 3) and add Ammonium Hydroxide Solution (SOLN B) drop by drop while stirring until the precipitate formed is ALMOST CLEAR. Stop when you reach this point. Note: Solution should appear slightly cloudy. Excess ammonia may cause a precipitate and impact results.

6. Place slides in ammonium silver solution (SOLN A & SOLN B) and stain at 37 – 40°C in oven or waterbath for 30 minutes or until sections become brown.

7. Place slides directly in Developer Working Solution for one minute or less. Note: Test should be performed on one slide initially, to avoid over developing and to determine a suitable incubation time.

continued on back

To prepare Developer Working Solution: add 8 drops of Developer Stock (SOLN C) and 8 drops of Ammonium Hydroxide Solution (SOLN B) to 50 ml of DI water, and stir.

8. Place slides in 1% Ammonium Hydroxide Solution (SOLN D) for 1 minute.

9. Wash slides in 3 changes of distilled water.

10. Wash slides in 5% Sodium Thiosulfate Solution (SOLN E) for 5 minutes.

11. Wash in 3 changes of DI water.

12. Dehydrate and clear through 95% ethyl alcohol, absolute alcohol and xylene.

13. Mount with Poly-Mount® Mounting Medium or other synthetic resin.

RESULTS

Neurofibrillary tangles and senile plaques-----black
Axons----------black
Nerve fibers---------- brown to black
Background----------yellow to brown

POSTIVE CONTROL – Brain tissue

PROTOCOL NOTES

1. Do not use any metal for this procedure.
2. Use glass staining dishes and glass stir rods for entire procedure. All glass must be acid cleaned or Alconox cleaned and rinsed with distilled water.
3. Use a glass thermometer, no mercury.
4. Developing time is critical so test a few slides and check microscopically to determine exact developing time.

REFERENCES

1. Bancroft, John D., and Marilyn Gamble. Theory and Practice of Histological Techniques. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 368-370.
2. Carson, Freida L., and Christa Hladik. Histotechnology: A Self-Instructional Text. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 202-205.

ORDERING INFORMATION:

Cat. #	Description	Size
25994-250	Bielschowsky Stain	250mL kit
25994-500	Bielschowsky Stain	500mL kit
08381-120	Poly-Mount®	120 mL
08381-940	Poly-Mount®	940 mL

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