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Part 9154
Revised December 2015

Bielschowsky, Lester King Modified Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9154A**
Solution A: Silver Nitrate 20%, Aqueous	250 ml
Solution B: Ammonium Hydroxide 28-30%, ACS	100 ml
Solution C: Developer	25 ml
Solution D: Sodium Thiosulfate 5%, Aqueous	250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:	
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Bielschowsky, Lester King Modified Stain Kit procedure is used to demonstrate nerve fibers, neurofibrils/tangles, senile plaques and axons. This stain can be instrumental in assisting in the diagnosis of Alzheimer disease and other neurological disorders.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 6-8 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. All glassware/plasticware must be acid cleaned prior to use.
    a. *See Procedure Notes #1 and #2.*
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
    a. *See Procedure Notes #3 and #4.*
3. Preheat container of Solution A: Silver Nitrate 20%, Aqueous in water bath to 37°C.
    a. *Warm 80 ml of distilled water in a separate container in water bath to 37°C for slide rinsing/holding steps.*
4. Place slides in 37°C Solution A: Silver Nitrate 20%, Aqueous for 15 minutes.
    a. *See Procedure Note #5.*
5. Remove slides from Solution A: Silver Nitrate 20%, Aqueous **(do not discard)**; hold slides in warmed distilled water.
6. Add Solution B: Ammonium Hydroxide 28-30%, ACS drop by drop into heated Solution A: Silver Nitrate 20%, Aqueous, swirling or stirring with a glass/plastic rod until precipitate disappears. **Do not go past this point.**
    a. *Approximately 10 ml of Solution B: Ammonium Hydroxide 28-30%, ACS will be required. More than 10 ml indicates ammonia is old and deteriorating.*
7. Place slides back into the Silver Nitrate Solution with added Ammonium Hydroxide in water bath at 37°C for 10 minutes.
8. Remove slides and hold in warmed distilled water. **(Do not discard Ammoniacal Silver Solution.)**
9. Add 1 drop of Solution C: Developer to the Ammoniacal Silver Solution, while swirling or stirring with a glass/plastic rod.
10. Return slides to heated Ammoniacal Silver Solution with added Developer, in water bath at 37°C for 5-15 minutes; average time of 6 minutes. Check slides microscopically at 3 minutes for development of neurons to dark brown. Follow with checks at 1 minute intervals to avoid silver over-development.
11. Rinse thoroughly in distilled water for 5 minutes.
12. Place in Solution D: Sodium Thiosulfate 5%, Aqueous for 5 minutes.
13. Rinse thoroughly in tap water.
14. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Senile plaques, neurofibrils/tangles	Dark brown to black
Neurons	Dark brown
White and gray matter	Yellowish brown
Nerve fibers, axons	Brown to black

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water. Cleaning glassware with bleach is not equivalent to acid washing.
2. Plastic (5500), plastic-tipped or paraffin coated metal forceps must be used with silver solutions to prevent precipitation of silver salts. No metals of any kind should come in contact with silver solutions. Only glass thermometers should be used.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining procedure.
5. Do not overuse silver solution. A maximum of 8 slides per 40 ml of Solution A: Silver Nitrate 20%, Aqueous is recommended.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

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.
3. King, Lester. "The Impregnation of Neurofibrils". *Yale Journal of Biology and Medicine* 14.1 (1941). 59-68.
4. Modifications developed by Newcomer Supply Laboratory.